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PROCEEDINGS

20TH SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

June 26-30, 1989

CHARLESTON, SOUTH CAROLINA

SPONSORED BY

THE SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE

IN COOPERATION WITH

WESTVACO CORPORATION

AND

CLEMSON UNIVERSITY

PROCEEDINGS OF THE

TWENTIETH SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

June 26-30, 1989

Charleston, South Carolina

Sponsored Publication No. 42 of the

Southern Forest Tree Improvement Committee

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PREFACE

The Twentieth Southern Forest Tree Improvement Conference was held at the Omni Hotel, Charleston Place, in Charleston, South Carolina. The conference was co-sponsored by Westvaco Corporation and Clemson University. There were 208 paid registrants representing 27 states and eight foreign countries.

A total of 57 oral presentations, seven invited and 50 voluntary, were given. All voluntary papers were evaluated by the SFTIC Committee and the Conference Committee to receive the $200 Tony Squillace Award. This award, initiated at the 18th SFTIC Conference, is given to the best oral presentation/written paper of the Conference. We are pleased to announce that the winners of the third Tony Squillace Award are Bailian Li, S. E. McKeand and H. L. Allen for their paper entitled "Early Selection of Loblolly Pine Families Based on Seedling Shoot Elongation Characters". Congratulations to all!

A total of 21 posters were displayed during the poster session which proved to be a popular event. The Baruch Foundation, Georgetown, South Carolina, generously provided a $100 award for the outstanding poster. The winners of this award are D. M. O'Malley, R. Sandozky-Dawes, T. Presnell, and R. R. Sederoff for their poster entitled "Characterization and Genetic Control of Cinnamyl/Alcohol Dehydrogenase in Loblolly Pine". Congratulations to Dave and his co-workers.

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OPENING SESSION

Twentieth Southern Forest Tree Improvement Conference

Charleston, S.C.
June 27 1989

State Forester's Address

Leonard A. Kilian
South Carolina State Forester

WELCOME TO CHARLESTON

I am delighted to be here today and especially honored that South Carolina is the host state for the Twentieth Southern Forest Tree Improvement Conference. Let me personally welcome each of you to South Carolina and express my sincere hope that you have a wonderful time here in Charleston during your meetings and during your free time.

The city of Charleston has a long and colorful history. As many of you know, Charleston was very much involved several years back in a couple of "Wars for Independence". Fort Moultrie and Fort Sumter each played a key role in defending a city and its people against attacking enemies.

The people of Charleston are a proud people and proud of their heritage. They continue to refer to Charleston as the "Holy City", or oftentimes as the "State of Charleston". The local culture is very unique and beautiful. We hope it is something you will want to capture: on film, through conversation, or with your pocket book. Charlestonians make no bones about it, they believe that here in Charleston is where the Asheley River on the West and Cooper River on the East come together to form the Atlantic Ocean... We hope that this week we too can all come together to enjoy this beautiful city and take home some wonderful memories of Charleston.

INTRODUCTION: PIONEERS IN FOREST TREE IMPROVEMENT

As I look around the room here this morning, I see the faces of some of the earliest workers in the forest tree improvement field; the so-called "pioneers" in the field. It was through your hard work and willingness to look into the future of forestry that tree improvement got its start. Let me tell it another way.

As you know, our newspapers recently were full of stories from China about students acting on a belief, a hope, or a vision for their futures. Re-

porters scrambled just to get a glimpse of students willing to act or express themselves for their beliefs in freedom; beliefs founded on hope and yet full of uncertainties. Those students took a chance. They stood to lose or they stood to gain.

That willingness to take a chance, although with a much greater measure of predictability and with something not quite as precious as human freedom, is just what many of you did several years ago in the tree improvement field. Your efforts were focused on improving the productivity of our forests through genetic engineering. You knew that if your calculations were correct, if the laws of nature held true, and if support could be won from the forestry community, your work would make a significant contribution towards increasing the productivity of our future timber resources.

The efforts you put forth did not go unnoticed. Today we can proudly say that those of you who took the lead, who dared to begin something new, are responsible for a well established system of forest tree improvement programs throughout the South. We are here this week to expand on those early seeds of knowledge and prepare for the future.

PROFESSIONALISM: EXPANDING LEVELS OF AWARENESS

This time last year I had the pleasure of addressing the Southern Nurserymen's Association meeting here in Charleston. At that meeting we spoke about our responsibilities of keeping ourselves up-to-date with the latest technological developments in our fields of expertise. Most of us tend to do a pretty good job of that. But we also talked about something even more broad than that, and that is, looking beyond the day-to-day task of growing trees and the technology associated with those jobs. We talked about nurserymen being a part of a bigger picture, about nurserymen becoming aware of changes taking place around them, and the changes in their forest industry.

A whole year has passed since then. And as they say, a lot of water has flowed under the bridge during that year. But as I look back over the past year I would have to say that the need to look beyond the everyday job

of growing tree seedlings, or in your case, of growing improved seed, is even greater. The influence of outside forces, forces within our own working environment, can have significant impact on how we do business, now and in the future.

You, as tree improvement specialists, at whatever level of responsibility, must expand your level of awareness and become knowledgeable of the changes that are taking place in the total environment in which you work and be prepared to make adjustments.

The environment in which we work has assumed increased importance and your organization's response to its environment may be crucial to its existence. As specialists, you must establish some method or approach that will enable you to do two things. First, you need to be able to monitor your working environment and the changes taking place. Second, you should be prepared to maintain and improve your performance given the changes taking place.

MONITORING YOUR WORKING ENVIRONMENT: YOUR INDUSTRY

Let's look at how we monitor our environment for a moment. We all read the daily newspaper, watch the evening news, and listen to the radio. These sources of information help keep us up to date on world events and local issues. Other sources of information are also available to help keep us up to date on forestry issues and changes that can have significant, and sometimes quite unpredictable effects on our organizations. We should review trade journals, association newsletters, government reports, statistics, and often-times "good ole street talk".

I strongly suggest, if you haven't already, that you join and actively participate in professional forestry organizations and even environmental groups. I personally had the opportunity earlier this month to participate in a Society of American Foresters meeting where four environmental groups presented their concerns and viewpoints on forestry issues of today. I must say it was an excellent forum for discussion and an enlightening one at that.

As tree improvement specialists, we "tap into" or monitor these sources

of information and try to identify trends in forestry. Then comes the hard part: forecasting changes and making adjustments in our programs and methods of operation accordingly.

Two important sources of information that we, in South Carolina, are using in this process of monitoring trends and forecasting changes in forestry are the South's Fourth Forest report and the Sixth Forest Survey report. In these reports we reaffirmed that:

1. Forest industry in S.C. is the State's third largest manufacturing industry. Timber is South Carolina's leading cash crop.

2. Net annual growth for pine has declined 28% in the last ten years. This is a significant reversal of the trend reported in the 1978 Forest Survey. We find that most of this growth loss is occurring on non-industrial private land (88%). There are four major causes of the decline in growth:

   -lack of adequate regeneration after harvest
   -increase in volume of mortality and cull trees
   -drop in radial growth on natural stands in the Piedmont.
   -conversion of timberland to cropland, pastureland, urban and other nontimber uses.

3. Pine timber removal is 103% of growth.

4. Twenty-three thousand acres of privately owned forestland are not regenerated after timber harvest each year. We have seen a significant increase in planting on non-industrial private land over the last ten years (143%).

5. Pine plantation acreage has increased tremendously. Projections for the next 30 years indicate an increase of 75% in area in pine plantations. Thirty percent of all timberland will consist of high valued pine plantations.

RESPONDING TO NEW INFORMATION

In response to this type of information and the implications it has for forestry in South Carolina we have taken the next step towards maintaining or improving our performance in tree improvement. We have moved forward with both simple or short-range plans and more complex or long-range plans. Again, we are monitoring our environment and making adjustments.

Many of you were affected by the passage of the Food Security Act of 1985; more commonly referred to as the Farm Bill which authorized the Conservation Reserve Program. (CRP is known outside the South as the Confederate Revenue Program) Among other things, this piece of legislation was designed to take highly erodible cropland out of production. Congress intended to change land use and in effect changed resource use including forestry. Those of you who were affected by this change in resource policy had to respond quickly to the sudden increase in the need for seed. You had to first become aware of the change and second, make the necessary adjustments in order to adapt to that change in your environment.

The Forestry Commission responded to CRP by increasing seed harvest and seed acquisition over a three year period to meet the increased demand for seedlings. We increased our total seedling production from 66 million seedlings in 1985 to 74 million seedlings in 1986. This past season we produced 80 million seedlings, 95% of which were from blue tag genetically certified seed. Unfortunately, we, along with several other southern states, overestimated the demand for seedlings and had somewhat of a surplus.

The Conservation Reserve program is an example of responding to an immediate need. Georgia(1), Mississippi(2), Alabama(3), and South Carolina(4) were impacted in a big way by the CRP signups. At last report these four southern states accounted for 73% of all CRP tree panting in the nation. The thirteen Southern Region states accounted for 93% of all CRP tree planting thus far. Final results of the eight sign-up indicate that the conservation reserve is now the largest land retirement program in history, with over 30 million acres enrolled nationwide. Our states did not have a lot of

time to monitor CRP's rather quick development or predict its eventual impact on our organizations.

A more complex or long-range view of things causes us to use our monitoring and planning skills for additional study. We invest much time and capital in planning for future needs. As tree improvement specialists you project annual seed requirements. Over the long term you must plan for additional land base and the monetary means for operational facilities; for progeny testing, and advanced generation orchards

The Forestry Commission moved forward in 1983 with long range plans to provide large quantities of genetically improved pine seed through the purchase of our second generation facilities at Niederhof Seed Orchard in Jasper County.

We began our tree improvement program in 1963 in cooperation with many forest industries and North Carolina State University. Similar tree improvement co-ops were established in Florida and Texas. Long range planning by such cooperators has helped to bring forth better trees for the South's next forest.

Niederhof Seed Orchard grafting work started in January of 1984 and I'm pleased to report that our second generation seed orchard is 99% complete today.

Today we are producing a limited supply of seed at Niederhof but we expect that by 1991 significant amounts of second generation seed will be produced. Our goal is to make our 1994 seedling crop 100% second generation material. We are proud of our progress in this area and we strive to continue to provide the best possible seed and seedlings for South Carolina's landowners.

CONCLUSION:

As we have discussed here this morning, and as I discussed a year ago with the Nurseryman's Association, we should take notice of the changes taking place around us, in our industry, and in our working environment and respond to these changes quickly and adequately.

We should also recognize those farsighted individuals who painstakingly research tree genetics and see-to-it that forestry keeps pace with the demand for forest products through genetic engineering. You folks are the modern day pioneers in forestry. Keep up the good work.

Thank you for your attention, and again, enjoy your stay in Charleston.

TREE IMPROVEMENT ACCOMPLISHMENTS IN THE SOUTH

A.E. Squillace 1/

Abstract.-- Tree improvement progress in the South during the past 40 years has been very significant, with 85 % of seedlings currently being planted coming from genetically-improved seed. Realized genetic gains in stand volume resulting from use of improved seed vary greatly, but average about 6 % for unrogued first generation loblolly and slash pine orchards and 17 % for rogued orchards. Appreciable gains in other traits, especially fusiform rust resistance, have also been attained. Many improved techniques, which are briefly discussed, assure that gains will continue to increase. The problem of decreased funding and manpower for research is pointed out along with possibilities for alleviating it.

Keywords: Forest genetics, tree improvement, southern pines, hardwoods

INTRODUCTION

It would be difficult to discuss our progress in all phases of tree improvement in a half-hour. Without attempting complete coverage, I shall first point out our progress in developing and employing superior trees and present evidence of genetic gains. Then I shall briefly discuss progress of some phases of research and techniques of special interest. Finally, I shall bring up the recent problem of decreased funding for research.

DEVELOPMENTAL PROGRESS

Accomplishments in developing superior trees during the past 40 years in the South have been tremendous. As of 1987 almost 10 thousand acres of seed orchards have been established (Table 1). Seeds from these orchards are producing 1330 million seedlings per year, sufficient for planting 1.9 million acres of forest land. This acreage planted with superior seedlings represents approximately 85 % of all trees currently being planted in the South.

Progress in the South has been considerably greater than in other major regions of the U.S. Data on production of superior seedlings by State nurseries in 1982 (Risbrudt and McDonald 1986) provide an approximate comparison. About 32 % of seedlings produced by southern state nurseries in 1982 were grown from genetically-improved seed, compared to an average of only 4.3 % for state nurseries of other regions.

1/ Retired plant geneticist, USDA Forest Service and Adjunct Professor, University of Florida, Gainesville, Florida.

Table 1. Development and utility of genetically-improved forest trees in the South as of 1987. 1/

Item	Organizations involved	Acres of seed orchards established as of 1987	Seedlings produced annually	Acres planted annually
	(no.)	(no.)	(millions)	(thousands)
NCSU	28	4000	630	900
WGFTIP	17	2050	350	500
CFGRP	15	2300	300	400
USFS	1	1400	50	60
Totals	2/	9750	1330	1860 3/

1/ Adapted from a report by Tim White in the Spring 1988 newsletter of the SAF Tree Genetics and Improvement Working Group.

2/ A total of 45 different organizations (one federal, eleven state, and thirty-three private) are involved. Some state and private organizations are members of more than one cooperative.

3/ This figure represents 85 % of all southern reforestation.

NCSU = North Carolina State University Tree Improvement Cooperative, N.C. State University, P.O. Box 8002, Raleigh, NC 27695.

WGFTIP = Western Gulf Forest Tree Improvement Program, Texas Forest Service, College Station, TX 77843.

CFGRP = Cooperative Forest Genetics Research Program, University of Florida, N-Z Hall, Gainesville, FL 32611

USFS = USDA Forest Service, Southern Region, 1720 Peachtree Rd, Atlanta, GA 30367

The excellent progress of tree improvement in the South is admittedly partly due to the extensive forest planting and demand for seeds. However, it is also undoubtedly due to the cooperative nature of our tree improvement activities, which was stimulated by the formation of the Southern Forest Tree Improvement Committee in 1951. About 33 private organizations work through one or more of the southern tree improvement cooperatives listed in Table 1. There are, in addition, 12 state and federal organizations involved. Organizations within each of the major forest areas of the South cooperate very closely, freely exchanging superior material (seeds, scions, etc.) and sharing technical knowledge. The progress is, of course, also due to the efforts of many individuals.

But I wish to mention five who were especially outstanding for both their promotional and research efforts, namely, Philip Wakeley, Keith Dorman, Bruce Zobel (unquestionably, the "Father of Southern Forest Tree Improvement"), Hans van Buijtenen, and Ray Goddard.

So much for developmental progress. How about the extent of genetic gains being made? Many researchers have predicted genetic gains on the basis of progeny tests. These are fairly suitable for some traits, such as fusiform rust resistance. But they usually are not considered highly reliable for estimating gains in wood volume per acre, because they do not adequately take into account effects of survival and competition. The most reliable estimates of such gains are based on large-block plantings of improved vs. unimproved seed using operational procedures (realized gain tests). These will be summarized later. But first note results from two exceptional predicted gain studies for loblolly and slash pine, based on many progeny tests and which give estimates for several orchard types in Table 2. Predicted gains in stand volume for unrogued and rogued first-generation orchards of loblolly pine (6.4 and 12.7 %, respectively) agree very well with those for slash pine (7.0 and 13.2 %). The estimate of no appreciable gain in fusiform rust resistance for unrogued first-generation orchards is typical--selections made in early years were not from highly-infected stands, and hence not resistant. But, the predicted gains of 18 and 30 % for rogued first-generation orchards and 1 1/2-generation orchards are very appreciable.

Reports of realized gains for loblolly and slash pines are summarized in Table 3. Gains in individual tree volumes are relatively consistent, varying from 2 to 14 % in unrogued orchards and from 14 to 21 % in rogued orchards. Gains in stand volume are highly variable, although the averages for unrogued and rogued orchards are about as expected, 6 and 17 %, respectively. The negative values are probably due to losses in survival. The losses reported by Tankersley (1983) were attributed to poor survival caused by high rust infection. The lack of gain for survival is expected in the first generation because we did not select for this trait (except that we did not select dead trees!). But losses in survival were frequent in rogued orchards also. Perhaps the reason for this is that natural selection has favored trees adapted to natural growing conditions, while our nursery and field planting techniques are far from natural. Perhaps we need to consider differences in survival more seriously in advanced-generation selection.

The very high gain in stand volume of slash pine (45%) reported by Kossuth et al. (1982) is exceptional. This is especially so in view of the fact that (1) the orchard contained only 9 clones, all of which were related to each other either as full- or half-sibs, and (2) that they were selected mainly for high gum yielding ability and to a lesser extent for rapid growth. The result tends to support previous work indicating a correlation between high gum yield and rapid growth. The apparent lack of inbreeding depression should be accepted with caution because very high wild pollen contamination was previously shown for this orchard. Further tests of seed from it would be desirable.

The Gladstone et al. (1987) report included other results of interest (not included in Table 3). They found that stand volumes were greater in single-family blocks than in mixed-family blocks, gains being 16 vs. 11 %, respectively. This apparent advantage of planting improved trees in single-family blocks should be tested further.

Table 2. Predicted superiority of loblolly and slash pine seeds from several clonal orchard types based on progeny test data.

Orchard type	Age	Gain (%) Stand volume	Gain (%) Fusiform rust resistance 1/
Loblolly pine (from Talbert 1982)			
First generation, unrogued	25	+6.4	---
First generation, rogued	25	+ 12.7	---
Slash pine (from Hodge et al., 1989)			
First generation, unrogued	20	+ 7.0	+ 2
First generation, rogued	20	+ 13.2	+ 18
One and one-half generation 2/	20	+ 18.0	+ 30

1/ $\frac{\text{(Percent infection of controls minus percent infection of orchard seed)}}{\text{percent infection of controls.}} \times 100$

2/ Orchards established with first-generation clones selected on the basis of progeny tests.

The average realized gains in stand volume for rogued and unrogued orchards, 6.2 and 17.1 %, respectively, are not greatly different from the predicted gains of Table 2. The lack of realized gain for rust resistance in unrogued first-generation orchards, with an appreciable average gain for rogued orchards, also agree fairly well with the predicted values of Table 2. In regards to this trait, White et al. (1989) showed a gain of 19% by selecting rust-free trees in highly-infected stands and also a gain of about 35 % by establishing seed production areas in highly-infected stands. Appreciable gains in other commercially important traits, such as wood specific gravity, stem straightness, oleoresin yield, and tall oil yield have also been shown.

Economic analyses show that, with even less than the average gains for stand volume reported above, tree improvement is an attractive investment for large forest land owners (Zobel and Talbert 1984, p. 451). Likewise, purchase of improved seed can be profitable for small landowners.

Table 3. Results of realized genetic gain tests for loblolly and slash pines.

Species	Stand age	Gains (+) or losses (-) as % of controls: Survival	Tree vol.	Stand vol.	Rust resistance	Authors
Unrogued First-generation Orchards (or Equivalent)						
Loblolly	8	-2	---	+2	---	Lowerts (1987)
"	4	-2	---	-3	---	"
"	8	+7	+7	+13	---	Gladstone et al. (1987)
"	22	+8	+8	+17	---	"
Slash	5	-7	+12	---	-4	Kraus & LaFarge (1984)
"	15	-3	+2	-1	-13	Tankersley et al. (1983)
"	6	0	+14	+9	+7	Lowerts (1986)
"	30	+6	+10	---	---	McReynolds & Gansel (1985)
Averages, both species		+0.9	+8.8	+6.2	-3.3	
Rogued First-generation Orchards (or Equivalent)						
Loblolly	6	-17	---	+20	+40	Lowerts (1986)
"	8	---	---	+3	---	Lowerts (1987)
	8	+11	+21	+34	---	Gladstone et al. (1987)
Slash	6	0	+14	+6	+23	Lowerts (1986)
"	10	-5	---	+45 [1/]	---	Kossuth et al. (1982)
"	4	+4	---	---	+34	"
"	4	-23	---	---	+33	"
Averages, both species		-5.0	17.5	17.1	+32.5	

[1/] This was an unrogued 2nd generation orchard, given 1/2 weight in computing the average.

Phenotypic selection of hardwood species in natural stands has usually been ineffective (Purnell and Kellison 1983). But, several authors have predicted gains for selection of families (or individuals) within progeny tests and establishing orchards (Table 4). Estimated gains look good, averaging about 10, 11, and 16 % for tree height, tree diameter, and stem volume, respectively. Several authors also reported superiority of selected families over controls in progeny tests (Table 4). Although these are not genetic gain estimates, they suggest that appreciable gains might be attained.

Table 4. Predicted genetic gains for first-generation rogued orchards and superiority of selected families[1/] for some hardwoods.

Species	Selection intensity	Age of test	Gains or superiority % Height	Dia.	Stem volume	Authors
			Predicted gains			
Sycamore	Best 21% families	5	4	6	---	Jourdain et al. (1983)
"	Best 3% individuals	5	14	18	---	"
"	Best 50% parents	8	22	24	24 [3/]	McCutchan (1983)
Green ash	Best 24% families	10	5	6	12	Stauder & Lowe (1983)
Sweet gum	Best 15% families	10	6	7	20	" (1985)
No. red oak	Best 50% individuals[2/]	13	7	7	9	LaFarge & Lewis (1987)
Sweet pecan	Best 20% families	6	29	--	--	Toliver & Zeringue (1983)
Averages			12.4	11.3	16.2	
			Superiority of selected families[1/]			
Sycamore	Best 10% families	10	22	24	59	Toliver and Dicke (1987)
"	Best 15% families	16	--	--	63	Byram et al. (1988)
Green ash	Best 15% families	15	--	--	40	"
Water-willow oak	" " "	15	--	--	158	"
Cherry-bark oak	" " "	10	--	--	62	"
Yellow poplar	" " "	10	--	--	18	"

1/ Superiority of selected families vs. controls in progeny tests.

2/ Conversion of a progeny test into a seedling seed orchard in this case.

3/ Gain based on tree dry weight rather than volume in this case. A gain of 43 % was also estimated for unrogued second-generation orchard using combined selection.

Four generations of breeding work with Eucalyptus grandis in south Florida has been tremendously successful. Realized gains of 100 and 83 % for stem volume and stand volume, respectively, have been reported by Meskimen (1983) and these were for a "commercial" level of selection intensity. A higher level of selection, "Premier," resulted in gains of 164 and 159 %, respectively. The majority of the overall gains resulted from selection in the first generation (ancestral trees, which perform poorly). Additional gains from selection in subsequent generations were nearer to those being obtained in southern pines.

PROGRESS IN RESEARCH AND TECHNIQUES

General Procedures for Tree Improvement

Although some false starts were made, I believe we have ended up with the most practical procedures for most of our species. In the beginning some people felt that the best way was to select superior trees and then develop vegetative propagules from them for forest planting. This was the feeling of researchers at Olustee when high gum yielding slash pines were selected in about 1940. People there at the time felt that in order to take advantage of these it would be necessary to develop techniques for propagating them vegetatively for use in forest planting. Hence, the emphasis was on research in rooting cuttings. Soon, however, some breeding work demonstrated that seeds from crosses between high gum yielders performed well, and the approach was accordingly altered.

Another approach considered was to produce hybrids for use in forest planting, and some work was done along this line. But the problem of mass production of hybrids and other problems were soon encountered and, as Dr. John Duffield once stated, "Species hybridization has ceased, at least for the present, to be an important approach in tree improvement." In the early 1950s the seed orchard approach was begun in the South. Some variations were practiced, such as development of seed production areas and seedling seed orchards, and these have been useful in special situations. Curiously, many researchers are working on the vegetative propagation approach again, but using more refined techniques, such as tissue culture (McKeand and Weir 1984). Commercial vegetative propagation has been successful with some species and may well become the technique of the future.

Geographic variation

I believe we have pretty well learned the extent and nature of geographic variation for most of our commercially-important pines, and work is progressing well with some hardwoods. However, I believe we have been somewhat lax in delineating "planting" or "deployment" zones. Terminology is somewhat confusing, but I am here referring to areas and/or sites for which separate strains of trees may be desired now or in the future. These delineations are preferably made on the basis of local tests which reveal the best seed for each zone. But, such factors as disease hazards, climate, soils, etc., can be used in preliminary delineations. One practical approach might be for each cooperator to test seeds from orchards of other organizations, especially those in different climates, along with their own for comparison. Such plantings could be done on an operational basis, to provide information on realized genetic gain as well. I believe intensive delineations may become more important in the future. Eventually genetic gains may reach a threshold and one way to increase them will be to develop strains for smaller planting zones. In any event, if additional

provenance testing is required for species that are currently widely planted, it will have to be done very soon. Pollen from the many plantations being established will often fertilize flowers on native trees, preventing forever the chance to establish reliable provenance tests for those species.

Our seed source studies have shown that local seed sources are usually safest but not always best. Seed from central positions of a species range and/or where climate is optimum will often perform better than seed from other areas, even if moved appreciable distances. For example, Lambeth et al. (1984) expect stand volume gains of 20 to 30 % from planting coastal North Carolina orchard seed in Arkansas and Oklahoma, compared to seed from local stands. Reasons for this situation may be that natural selection favors trees most suitable for performance under natural environments. But we, in planting trees, create a much different environment by growing seedlings in a nursery, preparing planting sites, etc.

Mating schemes and progeny testing

Most organizations are currently employing rather sophisticated mating schemes, such as disconnected half-diallels, for progeny testing and developing breeding populations (van Buijtenen and Namkoong 1983). These procedures have several good advantages, such as providing opportunities for capitalization of specific combining ability, increasing genetic variation and giving maximum gain per generation. Some recent reports, however, give good arguments for using simpler schemes, such as polycrossing, single-pair matings, and even use of wind-pollinated seed, which can shorten generation intervals and produce greater gains per unit of time (Cotterill 1986). Zobel and Talbert (1984, p. 430) hypothesize that a 25 % increase in genetic gain per unit of time can be made by reducing generation interval from 20 to 16 years. Possibilities for decreasing generation intervals by growing families at very close spacing over a short period of time are promising (Franklin 1983). Likewise, new flower stimulation procedures (Greenwood 1983) can greatly shorten generation intervals.

Along this line, I am wondering if progeny testing of second-generation selections is necessary. Techniques for adjusting for environmental gradients in plantations have been developed (Bongarten and Dowd 1987). Likewise, adjustments for extent of competition can also be made (Smith 1987; Land and Nance 1987). Thus, if a candidate selection is phenotypically superior after adjustment for these factors, and its siblings are at least moderately superior, and its parents are genetically superior, is progeny testing worth the effort? Omission of progeny testing would, or course, reduce generation intervals and would decrease costs considerably. Selections would still need to be interbred in order to establish the next generation, but this would be a relatively small job compared to breeding for progeny test purposes. The question certainly seems worthy of study.

Seed orchards

Many new seed orchard techniques have been or are being developed, particularly in pollen management (Franklin 1981). For example, seed yields and genetic gains can be increased and wild pollen contamination reduced by supplemental mass pollination, a technique which has been shown to be operational

(Bridgewater et al., 1987; Blush 1987). Movement of seed orchards southward is another promising technique for increasing seed yields and for avoiding contamination (McKinley 1987).

Fusiform rust resistance

Techniques for developing rust resistance have progressed rapidly after learning that phenotypic selection in the wild was effective only in highly-infected stands. Several new procedures for early assessment of resistance are now available or are forthcoming. Establishment of the Rust Resistance Screening Center of the USDA Forest Service at Asheville, North Carolina, was a very valuable development. In vitro techniques are promising (Frampton, et al., 1985) as well as indirect selection using monoterpenes [Squillace et al., 1985; Michelozzi et al. (1989)]; and use of early symptoms of the disease (Layton 1985). Delineation of rust hazard patterns (Phelps 1974, Squillace 1976) has been of great help in breeding strategy. Byram et al. (1987) recently suggested that loblolly pine selections should be mated with highly-susceptible trees to test for resistance because the trait seems to be controlled by dominant genes. This agrees with some unpublished work I did with slash pine, which showed that a large part of the variation among families of known parentage could be explained by assuming two dominant genes.

THE PROBLEM OF DECREASING FUNDING

During the 1980s both federal and nonfederal funding for forest research decreased by roughly 25 % (Giese 1988), resulting in high losses in manpower for tree improvement work. What can we do about this? Certainly we should try to avoid further losses. Better information on realized gains, especially for rogued and advanced-generation orchards will help. But perhaps we should also think about what can be done if further losses occur. The suggestions of using simplified mating and progeny testing techniques, mentioned earlier, could be considered. A methodology suggested by Franklin (1986) for use with minor species could be considered for wider application. It entails use of a single plantation per generation, which serves as a progeny test of trees selected in the previous generation, a basis for the selection and breeding for the base population of the next generation, and finally as a seedling seed orchard. An even simpler procedure was suggested for minor species by Squillace (1979). It involves the establishment of seed production areas in regions of appreciably different environments in planted stands of each generation, providing for both artificial and natural selection. The latter type of selection would be important where environments, such as virulence of fusiform rust, may change with time.

CONCLUSIONS

Our tree improvement efforts of the past 40 years have been very successful in developing superior trees with appreciable genetic gains. The many new and better techniques being developed, along with the continued high degree of cooperation among organizations involved, will help insure continued success. But we must also try to avoid further cuts in funding and may have to start employing shortcuts.

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INTER-GENOTYPIC COMPETITION IN FOREST TREES AND ITS IMPACT ON REALIZED GAIN FROM FAMILY SELECTION

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Abstract.--The impact of inter-genotypic competition on realized gains from tree improvement programs remains largely unknown. Empirical results for individual tree traits demonstrate generally non-significant family x plot type (e.g., block, row, and non-contiguous) interactions and moderately strong, positive, family rank correlations between pairs of plot types. Therefore, results to date seem to indicate that average family performance is stable across a range of levels of inter-family competition. Inter-family competition either increased or decreased family variance, hence the estimate of additive genetic variance. Estimated genetic parameters, including genetic gain, for the same population tested in both block plots and row or non-contiguous plots bear little resemblance. Therefore, data from row plot or non-contiguous plot tests should not be used to estimate genetic parameters, including realized genetic gain.

Testable hypotheses are developed which integrate current experimental results to provide a framework for future research regarding the influence of inter-genotypic competition on realized gain from selection.

Keywords: additive genetic variance, growth and yield

INTRODUCTION

Competition is a dominating factor in the development of forest stands. This fact has been known for well over a hundred years and has provided the basis for many of the silvicultural techniques used by foresters. Inter-tree competition acts to shape the individual tree (Harper 1977), to induce mortality (Harper 1977; Drew and Flewelling 1979) and to influence the shape of population distributions (Mohler et al. 1978). As forest geneticists explore the effects of selection and breeding on trait improvement, the confounding effects of inter-genotypic competition become important (Ford 1976; Cannell 1984).

The focus of this paper will be on the importance of inter-genotypic competition to the efficiency of selection of superior families and the subsequent realized gain. Competition in this sense is defined as the stress which is placed on a plant by the phenotype and spatial arrangement of the surrounding plants (Hinson and Hanson 1961). Research results regarding inter-genotypic competition in forest trees up to 1980 were reviewed by Adams (1980).

Competition can result in changes in the population mean or in phenotypic or genetic variance (Hamblin and Rosielle 1978). In their study with rice (Oryza sativa L.) and barley (Hordeum sativum L.), Hamblin and Rosielle (1978) found relatively small changes (largest effect was an 11.5% increase) in the means due to competition between varieties; however, the additive and dominance genetic

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variances were strongly affected. The competitive variance was: 1) up to twice the size of the additive genetic variance and of the same or opposite sign and 2) up to half the size of the dominance genetic variance and of opposite sign. Therefore, genetic variance can be either inflated or suppressed by competitive variance. Hamblin and Rosielle concluded that estimates of genetic parameters obtained in mixtures may have little relevance for pure cultures. Sakai (1955), in his work with several agronomic species, demonstrated that competitive pressure increases as the density of the plants becomes larger (e.g., closer spacing). For simplicity, the research results in forestry pertaining to the effects of plant density on genetic parameters will not be reviewed in this paper.

Genetic test design (e.g., pure block plots, row plots or non-contiguous plots) and deployment strategy of improved seed remain in a state of flux for forest trees. In general, organizations are using small plots (e.g., 1 to 5 trees) in either rows or in non-contiguous fashion (Figure 1) for progeny testing; yet improved seed is being deployed in reforestation as both large (50 acres or more) half-sib family blocks and bulk mixtures of families in varying proportions. Forest geneticists are increasingly concerned about the disparity between the testing and selection environment and the deployment environment (e.g., Cannell 1979, 1984; Gladstone et al. 1987). Traditionally, progeny test design and subsequent selection in forestry have emphasized individual tree traits (e.g., means for height, d.b.h., and volume); yet the production unit is volume per unit area. Little emphasis has been placed on rectifying the inconsistency between selection unit and production unit. The current paper will summarize research results to date, draw some conclusions regarding the effects on inter-genotypic competition on realized gain from selection for both individual tree traits and per unit area traits, and formulate testable hypotheses to guide future research.

INDIVIDUAL TREE TRAITS

Estimation of genetic value and genetic variances is a statistical as well as a genetic problem. The statistical issues center around sampling theory to estimate environmental effects precisely and separate these effects and their interactions from genetic effects. Environmental effects can be divided into 3 broad classes: location differences, patchiness or gradients within a location, and microenvironmental effects among trees within small areas. Progeny test design has been optimized to sample each of these levels of environmental variation for the efficient estimation of family means and family ranking (e.g., Lambeth et al. 1983; Cotterill and James 1984).

Impact on family means

Detection of family mean differences appears to be optimized by using 10 to 20 individuals per family at each test location (Cotterill and James 1984) with plot sizes of 1 to 5 trees (e.g., Johnstone and Samuel 1974; Cotterill and James 1984) in which the trees are arranged in a non-contiguous plot fashion (e.g., Lambeth et al. 1983; Cotterill and James 1984). There is a clear tradeoff between plot size and number of families versus replication size. Maximum available replication size subsequently depends upon the patchiness of the environment at each location, which is clearly a limiting factor. Enough locations must be sampled to detect significant macroenvironmental differences

as well as potential family x location interactions.

Although the optimum design, as stated above, provides for efficient estimation of family means, no consideration is made of the influence from inter-family competition. The question of level of influence pivots on the issue of whether trees with more similar genotypes (sibs) will compete more, the same, or less than trees with less similar genotypes (unrelated trees) (Mather 1969). A continuum exists in the level of inter-family competition, which ranges from intense (single-tree plot or non-contiguous multi-tree plot) to no inter-family competition (pure family block plot with interior border rows)(Figure 1). The row plot represents an intermediate level of inter-family competition since an individual tree may compete with 1 sibling and individuals from 3 different families (row end position) or with 2 siblings and individuals from 2 different families (mid row position).

Researchers have used 2 basic approaches to establish the influence of various levels of inter-family competition on the determination of family means or ranks. The first approach involves planting the same families in various plot configurations and then comparing the means by analysis of variance or comparing the family ranks between plot configuration treatments by rank correlation. The second approach involves some type of statistical adjustment procedure to equalize the level of inter-family competition. Comparisons are made between: 1) results of an analyses of variance before and after data adjustment or 2) family ranks between adjusted and unadjusted treatments using rank correlation.

Block plot	Non-contiguous plot	Row plot
**********oooooo	-----	-----
**********oooooo	-o++-	-*+o-
**********oooooo	-*+o-	-*+o-
**********oooooo	-**o-	-*+o-
**********oooooo	-o*+-	-*+o-
	-+*o-	-*+o-
	-oo*-	-*+o-
	-+*+-	-*+o-
	-*o+-	-*+o-
	-o+*-	-*+o-
	-----	-----

Figure 1. Layout of 1 replication each of 3 plot types: block, non-contiguous, and row. Each replication contains 3 families (represented by: *, +, and o) with 9 individuals per family. The block plot has a border row of the same family surrounding the 9 measurement trees while the replications of the non-contiguous and row plots contain a single border row (represented by: -) around the outside.

Using different plot configurations

Closely spaced seedling trials and regularly spaced field trials have been used to examine the effects of varying levels of inter-family competition on family means or ranks. Adams et al. (1973) and Tuskan and van Buijtenen (1986) utilized seedlings, less than 1-year-old, of loblolly pine (Pinus taeda L.) in competition studies. The studies were similar in approach in designating a subject tree, of a certain family, which was surrounded by either 5 (Tuskan and

van Buijtenen 1986) or 6 (Adams et al. 1973) seedlings from the same family (pure combination) or differing numbers of seedlings from another family (binary combination). Adams et al. (1973) tested 4 full-sib families using a diallel arrangement (totaling 12 pure or binary combinations) while Tuskan and van Buijtenen (1986) tested 5 open-pollinated families using a diallel arrangement (totaling 20 pure or binary combinations). In both studies, the families responded differently to competition. The family means depended on the proportion of the surrounding seedlings which were from the competing family (dose level) and the specific binary combinations (e.g., some families interacted while others were neutral in combination). The authors in both studies noted growth performance of family combinations which were overcompensatory (treatment performance exceeds the weighted mean of the 2 pure family performances), complementary (the treatment performance is balanced-what one family loses the other one gains), undercompensatory (treatment performance is significantly less than the weighted mean of the 2 pure family performances), or neutral (families coexist with little effect on each other).

At least four studies have been reported in the literature which compared the field growth of the same families in various plot configurations. Unfortunately, the results have been somewhat conflicting in that the generally positive relationship between family means or ranks is consistent, yet the closeness of the relationship varies.

In their loblolly pine study, Williams et al. (1983) found no significant interaction between family and plot type for height or individual tree volume at 4 years. Their study included pure family block plots and a mixed plot of 4 seedlings, planted in non-contiguous fashion, from each of the families. The trees in the study were only 4 years old and planted at a 9.8 ft (3 m) square spacing; however they had grown rapidly, and the consistency of treatment performance at age 8 (Gladstone et al. 1987) supports the implication of at least moderate competition effects at age 4.

Rockwood (1983) reported on 3 studies with slash pine (Pinus elliottii var. elliottii Engelm.) which compared the same families in block plots with plots in which the measurement tree was surrounded by 8 trees from the other families (like a non-contiguous plot). Each of the 3 studies contained both plot types at the same location. There was no significant difference in family height or diameter at breast height (d.b.h.) between the plot types after 4 (2 studies) or 5 (1 study) years in the field, which agrees in principle with Williams et al. (1983). As with the study by Williams et al. (1983), these trials were somewhat young to be assessing competition effects; however the trees averaged about 3 ft of annual growth and had probably started to compete for resources.

In his report of a group of 10-20 year old loblolly pine studies, Morrow (1983) compared growth differences for 3 sets of families planted in both block plots and row plots in progeny tests. A single plot type was represented at each of several locations. For 2 sets of families, each of the plot types was represented by 2 locations while the third set of families had 2 locations of the row plot type and only 1 location of the block plot type. Some locations within a set of families varied in age. No significant plot type x family interaction occurred with any of the 3 sets of families for height, d.b.h., or individual tree volume. Spearman rank correlations for family rankings between

block and row plot types were positive in all cases (Table 1) and were significant for each family set and trait for which family differences were significant in the analysis of variance. The analyses of variance for family differences for height and d.b.h. for the third family set were non-significant; similarly, the associated rank correlations were non-significant. Therefore, the results of the analysis of variance in this study compare favorably with those of Williams et al. (1983) and Rockwood (1983); however, the rank correlations reveal that some rank change, although not statistically significant, was occurring.

Table 1. Spearman rank correlations between family mean rankings for: 1) block plots and row plots (Morrow 1983), and 2) block plots, row plots, and non-contiguous plots (Hart 1986) for height, d.b.h., and individual tree volume for loblolly pine progeny tests.

	Rank correlations		
Plot comparison	Height	D.b.h.	Volume
1. Morrow 1983			
analysis 2 - block vs. row	0.83^{**}	0.72^{**}	0.87^{**}
analysis 3 - block vs. row	0.63^{*}	0.65^{*}	0.66^{*}
analysis 4 - block vs. row	0.12^{NS}	0.24^{NS}	0.74^{*}
2. Hart 1986			
Block versus row	0.55^{NS}	0.02^{NS}	0.07^{NS}
Block versus non-contiguous	0.62^{NS}	0.36^{NS}	0.31^{NS}
Row versus non-contiguous	0.76^{*}	0.52^{NS}	0.52^{NS}

^{NS} not significant at p=0.05; * significant at p=0.05; ** significant at p=0.01

Hart (1986) analyzed differences in family mean performance for an 11-year-old loblolly pine trial which included the same 8 families in 3 plot types: block, row, and non-contiguous. Family rank correlations between pairs of plot types were used to examine family differences resulting from an interaction with level of inter-family competition. All family rank correlations between pairs of plot types were positive although only the rank correlation for height between the row plot and the non-contiguous plot was statistically significant (Table 1). For height, d.b.h., and individual tree volume, the rank correlations between the row plot and the non-contiguous plot types were consistently the largest (range of 0.523 to 0.761); and the correlations between the block plot and the row plot types were consistently the lowest (range of 0.023 to 0.547). Among the 3 traits, height produced the highest correlations. One family was neutral, and 2 families exhibited positive yield responses to increased levels of inter-family competition. The rank correlations given by Hart (1986) were generally lower and less statistically significant than Morrow's (1983). One contributing factor was that Hart's trial only included 8 families which was lower than any of Morrow's trials.

Using adjustment procedures

A variety of data adjustment procedures have been used in an attempt to equalize the effects of inter-family competition on family mean growth and family ranking. Five studies are reviewed in this paper: 1 used the average of the surrounding plots, 1 used differences in subject tree and competitor heights,

while 3 used some measure of available growing space for data adjustment.

In their radiata pine (Pinus radiata D.Don) study, Correll and Anderson (1983) used the mean of the 2 adjacent row plots to adjust each family row plot for local environmental effects within the progeny test. Although the adjustment resulted in improvement of the analysis of variance results, there was little change in the ranking of the families.

Angle of height difference between a subject tree and its neighbors formed the basis for a competition adjustment model used by Magnussen (1989) on a closely-spaced, single-tree plot trial of jack pine (Pinus banksiana Lamb.) for ages 1-5. Height, through age 5, displayed little effect from inter-family competition; however family-mean basal area and volume were strongly influenced and required significant adjustment. Comparing unadjusted and adjusted data, the difference between the fastest and slowest growing families declined by 1/3 for basal area and 1/2 for volume. Despite the reduction in the ranges of family means for basal area and volume, the family-mean rank correlations between adjusted and unadjusted values were 0.998, 0.792, and 0.818 at age 5 for height, basal area, and volume, respectively. These results concur with those of Correll and Anderson (1983) in showing only slight changes in family rank following data adjustment.

The concept of effective growing space utilized by each tree was used by Pinker (1982) to adjust individual tree volume for differential competition in an 11-year-old eastern white pine (Pinus strobus L.) and a 14-year-old Virginia pine (Pinus virginiana Mill.) progeny tests. Both tests utilized row plots. The area of effective growing space assumes the shape of a polygon and was estimated in this study as a function of the volume of the subject tree and the volume of each competitor tree and distances between the subject tree and each competitor. Spearman rank correlations between adjusted and unadjusted family means were highly significant and reached 0.759 and 0.631 for white pine and Virginia pine, respectively. Therefore, this adjustment procedure had little effect on the ranking of the families within each species, agreeing in results with Correll and Anderson (1983) and Magnussen (1989).

In their loblolly pine study, Nance et al. (1983) employed the same concept as Pinker (1982), with a slightly different formula, to calculate the area potentially available (APA) to each subject tree. Diameter at breast height and the distance apart of the subject tree and its competitors were used to calculate the APA as an irregularly shaped polygon. Furthermore, the polygon was subdivided into components due to competitors, from either the same family as the subject tree or different families. The data set employed in the analysis arose from a Nelder's design study with row plots of 8 families. Height, d.b.h., and live crown length were measured at ages 7 and 8 years. Multiple regression models were developed which included basal area growth (BAG) in the seventh year as the dependent variable and a variety of components (e.g., tree measurements and APA) as the independent variables. APA was the single best predictor of BAG. In addition, the average APA of the family of the subject tree (APA_i) added significantly to basal area of the subject tree, height of the subject tree, crown length as a ratio of total height of the subject tree, and APA of the subject tree as predictors of BAG. Neither the general relative influence (at the family level) of the competitors or the specific relative influence between the competitors and subject tree added significantly to the model predicting BAG.

These results indicated that families differed in their use of available growing space for basal area growth; yet after considering APA, the family effect of the competitor was unimportant. Two of the 8 families expressed significantly different APA_i : 1 family was more and 1 family was less efficient than average in using the available growing space.

In further analysis, Hart (1986) used the procedures developed by Nance et al. (1983) to examine his loblolly data from the 3 plot type study discussed previously in this paper. He calculated the APA for each tree using age 9 data. Judging by average APA, the block plots exhibited the highest amount of inter-tree competition (79.6 ft^2) with row plots being intermediate (80.7 ft^2) and non-contiguous plots displaying the least (81.1 ft^2). Although there was little actual difference in average APA among the plot types, the range of average family APA within the non-contiguous plot type was about 3 times that for the block plot type. Therefore, the more intense inter-family competition inherent in the non-contiguous plot type resulted in much more variable APA among families. Families differed significantly in their basal area growth (age 9 to 11)(BAG) per unit of APA in the non-contiguous plot type but not in either the block plot or row plot types. However, both row plot and non-contiguous plot types displayed significant volume growth differences per unit APA among families while no family differences were apparent in the block plot type. Therefore, even though the APA was slightly less for the block plot type, the level of competition among the families was more uniform for the block plot type; and competition-induced family differences in volume or basal area growth per unit APA were not apparent in the block plot type. There was not a strong relationship between either volume growth per unit APA or BAG per unit APA and volume production as a percentage of anticipated production; so the most efficient users of APA were not always the highest volume producers.

Summary

Family means or ranking for individual tree traits, especially for height, appeared to be more or less unaffected by varying levels of inter-family competition. Although the relationships between family means or ranks between different plot types or unadjusted versus adjusted data were not perfect, they were correlated strongly enough to indicate little negative impact on realized gains from family selection (e.g., breeding value was estimated with reasonable precision). This result was due, at least in part, to a large number of data points contributing to the mean. Randomization procedures assured that the individuals within a family sampled the entire range of competitive interactions. As the number of replications increases, the assumption is more closely met that the effects of interactions among families is at random, hence unbiased estimates of family means are obtained.

The significance of family differences varied by plot type; however, this finding may be due to changes in real differences among treatment means or the size of the error variance. Therefore, the family means may vary slightly among plot types; yet changes in the error variance, by plot type, may alter the significance levels. The effects of changes in inter-family competition on variances are discussed in the next section.

Impact on family and block x family variances

One possible objective of progeny testing is the estimation of genetic and environmental variances. Much research has been devoted to constructing designs which provide accurate estimates of these variances (e.g., Namkoong 1979).

Researchers have recognized for some time that inter-genotypic competition may, and probably does, bias estimates of genetic variances (e.g., Sakai 1955; Sakai and Mukaide 1967). Theoretically, the variance among half-sib families (σ_{HS}^2) estimates 1/4 of the additive genetic variance; while the variance among full-sib families estimates 1/2 of the additive genetic variance, 1/4 of the dominance genetic variance, and differing amounts of epistatic components of genetic variance (Becker 1984). These models of gene action do not acknowledge the presence of changes in family variance due to competition and would apply only to family block plots. With other plot types which encourage the interaction among families, a variance term (σ_{comp}^2) must be included in the model to account for either added or subtracted variance due to inter-family competition.

Using different plot configurations

Three published studies have documented the effect of different plot configurations on family and block x family variances. When the same families are included in 2 or more plot types within the same replication, an estimate of σ_{comp}^2 can be obtained by calculating the difference between the estimates of family variance (e.g., from block plot to row plot type).

Williams et al. (1983) found that the family variance for height was only 52% as large (σ_{HS}^2 = 0.0115; non-significant at p=0.05) for the block plot type as compared to the non-contiguous plot type (σ_{HS}^2 = 0.0223; significant at p=0.01). The added variance due to increased inter-family competition (σ_{comp}^2 ; Table 2) would result in inflated estimates of additive genetic variance. The inflation was 94% for height and 147% for volume. They analyzed plot mean data; therefore, the block x family variance served as the error variance and included 1/n of the sampling variance (within plot variance). The block x family variance for height was twice as large for the block plot type (0.04) as for the non-contiguous plot type (0.02). Hence increasing inter-family competition appeared to increase the family (e.g., genetic variance) variance while decreasing the block x family variance. Family-mean heritability for height increased from 0.43 to 0.76 from the block plot to the non-contiguous plot, respectively, while for individual-tree volume, it increased from 0.34 to 0.70 from the block plot to the non-contiguous plot, respectively.

In their study, Lambeth et al. (1983) provided family and block x family variance components for both the row and non-contiguous plot types. Generally, the row plot type provided family variance components that were 13% higher than the non-contiguous plot type. In 4 of the 8 trials, the family variance was larger for the row plot type; while in 3 of the 8 trials, the non-contiguous plot type resulted in a larger family variance. Competitional variance (σ_{comp}^2), as a ratio of family variance for the row plot type, averaged a reduction of -0.05 (Table 2); however, the effects among the individual tests ranged from 1.08 to -0.75. Hence the estimates of genetic variance could be affected tremendously by inter-family competition. The non-contiguous plot type resulted in block x

family variance estimates which were essentially 0.0 while the row plot type estimates were even larger than the family variance components. In this study, the block x family variance did not include the sampling variance (within plot variance); that is, individual tree data were analyzed. Therefore, the family variance decreased slightly with increasing inter-family competition (as opposed to the findings of Williams et al. 1983); yet the block x family variance decreased markedly, as Williams et al. (1983) found.

Table 2. Inter-family competition variance (σ_{comp}^2)[a] estimated from loblolly pine studies which included the same half-sib families in various plot types.

Study	Block to row		Block to non-contig.		Row to non-contig.	
	σ_{comp}^2	$\sigma_{comp}^2/\sigma_{HS}^2$	σ_{comp}^2	$\sigma_{comp}^2/\sigma_{HS}^2$	σ_{comp}^2	$\sigma_{comp}^2/\sigma_{HS}^2$
1. Williams et al. (1983)						
height			0.0108	0.94		
volume			2.46	1.47		
2. Lambeth et al. (1983)						
height						
Test-set						
1-1					-0.010	-0.22
2-1					-0.005	-0.07
3-1					0.011	0.14
4-1					0.013	1.08
5-1					-0.036	-0.75
6-1					0.003	0.25
7-1					-0.010	-0.59
8-1					-0.003	-0.20
average					-0.004	-0.05
3. Hart (1986)						
height	0.1412	---	0.2369	---	0.0957	---
d.b.h.	0.0039	1.26	0.0207	6.68	0.0168	5.42
volume	29.57	0.59	200.01	3.97	170.44	3.38

[a] Assumes that the family variance component derived from pure family block plot types expresses genetic variance that is free from inter-family competition variance; all other plot types confound family variance with inter-family competitional variance.

Hart's (1986) study provided an excellent comparison of variance component estimates among 3 plot types: block, row, and non-contiguous. The pattern was virtually the same for height, d.b.h., and individual tree volume. Family variance increased from block plot to non-contiguous plot with row plot being intermediate (Table 3). Family differences were not significant for the block plot type for any of the 3 traits or for the row plot type for either d.b.h. or volume while they were significant for the non-contiguous plot type for all 3 traits (Table 3). Competitional variance (σ_{comp}^2) always increased the estimate of genetic variance (Table 2). The inflation ranged from 59% to 668%. The block

x family variance included sampling variance (within plot variance) in this study A pattern of decreasing variance from the block plot to the row plot and finally to the non-contiguous plot was expressed for the block x family variance (Table 3). Therefore, the pattern was clear in this study that family variance increased and block x family variance decreased with increasing inter-family competition. Family-mean heritabilities increased dramatically from the block plot to row plot to non-contiguous plot type, more than doubling from the block plot to the non-contiguous plot type (Table 3). This pattern of change for both variances and heritabilities mimics the results of Williams et al. (1983).

Table 3. Family and block x family variance components and family-mean heritability[a] for height, d.b.h., and volume derived from a loblolly pine progeny test which included block plot, row plot, and non-contiguous plot types (adapted from Hart 1986).

	Family variance			Block x family variance		
Trait	block	row	non-contig.	block	row	non-contig.
Height	$<0^{NS}$	0.1412^{*}	0.2369^{**}	1.9253	0.4968	0.4294
D.b.h.	0.0031^{NS}	0.0070^{NS}	0.0238^{**}	0.0592	0.0498	0.0378
Volume	50.42^{NS}	79.99^{NS}	250.43^{**}	722.21	551.58	483.30

	Family-mean heritability		
	block	row	non-contig.
Height	0.00	0.63	0.77
D.b.h.	0.24	0.46	0.79
Volume	0.29	0.47	0.76

^{NS} not significant at p=0.05; * significant at p=0.05; ** significant at p=0.01

[a] $h_F^2 = \dfrac{\text{family variance}}{\text{family var.} + \dfrac{\text{(block x family var.)}}{\text{\# blocks}}}$

Using adjustment procedures

The influence of inter-family competition on family variance has been examined by means of data adjustment procedures. Two authors, using either height difference adjustment or effective growing space adjustment, demonstrated clear differences in family variance (i.e., additive genetic variance estimates) between unadjusted and adjusted data.

In his jack pine study, Magnussen (1989) showed a small, non-significant difference in family variance (σ_{HS}^2) for height for ages 1-4 and a slightly larger, yet non-significant, difference at age 5. However, for basal area at ages 4 and 5, the family variance for unadjusted data was about 200% of the size of the adjusted data value. At age 5, family variance for unadjusted volume was 700% the size of the family variance for the adjusted data. Therefore, family variance and subsequent estimates of additive genetic variance may be inflated only slightly for unadjusted height in single tree plot experiments; however, inter-family competition, as evidenced by adjustment procedures, strongly inflates family variance for basal area or volume in such studies.

Pinker (1982) utilized an adjustment procedure to investigate the amount

of bias, from inter-family competition, on family variance (σ_{HS}^2). He found that the family variance for adjusted individual-tree volume was only 22% and 9% the size of family variance for unadjusted volume for Virginia pine and white pine, respectively. The block x family variance was estimated as a separate parameter from the sampling variance or within-plot variance (i.e., individual tree data were analyzed). While family variance decreased from unadjusted to adjusted data (as with Magnussen 1989), the block x family variance increased from unadjusted to adjusted data. Therefore, the pattern of change, from removing the effects of inter-family competition by data adjustment, mimics the pattern that results from comparing the same families in row plots or non-contiguous plots with block plots (Table 3).

Summary

Among-family variances seem to be strongly impacted by varying levels of inter-family competition. This finding with forest trees parallels the findings of Hamblin and Rosielle (1978) who noted large changes (both positively and negatively) in genetic variances due to inter-genotypic competition in rice and barley. Genetic parameters (e.g., additive, dominance, and epistatic effects, heritability, and genetic gain), which are determined from variance component estimates, are subsequently biased. Additionally, variance due to competitional effects (σ_{comp}^2) is trial specific (e.g., specific families and environmental effects) and therefore cannot be wholly transferred to operational plantations as part of genetic gain (although a remnant of inter-family competitional variance will remain). This is especially true since row plot trials are often used to project genetic gain in operational plantations with either large pure family blocks or seed orchard mixtures of families, including families not combined in the original progeny tests and families in different proportions.

To provide valid estimates of realized genetic gain, trials must be established with a plot type and family components that closely mimic the operational plantation procedure. If pure family blocks are planted operationally, then they should be used in the realized gain trial. If mixed plots (e.g., like non-contiguous plots) are used operationally (e.g., orchard bulk lot), then the trial should use that plot type. Since block plots avoid inter-family competition, then the same trial could serve as both a progeny test and a realized gain trial for an organization which operationally planted large family blocks. Families which are not selected, based on progeny test results, would be purged from the data set prior to re-analysis for a realized gain estimation.

STAND LEVEL TRAITS

Yield per unit area is the factor of major concern to forest managers. Although the mean height, d.b.h., and individual tree volume are positively related to yield per acre, other factors such as site index, age, stand density, and quadratic mean diameter are more closely related to yield per acre (Clutter et al. 1983). Of these stand level factors, site index (mean height of the dominant-codominant trees at a base age) appears to be most amenable to genetic manipulation.

Nance and Wells (1981) first fit site index curves to seed source data. They demonstrated the more reliable behavior of site index rather than mean height to detect seed source differences, especially when unusual events (i.e.,

insect damage) reduce stocking levels below normal. Additionally, Nance and Bey (1979) explored the use of a growth and yield model, driven by source-specific site index curves, to project per acre yield in simulated genetically improved stands. Finally, Knowe and Foster (1989) fit site index equations to individual loblolly pine families, growing in block plots, and then utilized the specific equations to simulate per acre yield with a stand simulator. The simulated per acre yields compared very closely to actual yields to age 15 (age of oldest data). Assuming a continuingly accurate prediction, the authors predicted yield to age 25 and then estimated genetic gain from selection of the top families. Through age 15, the predicted and realized genetic gains in per acre yield were very close. Therefore, prediction of realized genetic gain in per acre yield appears feasible when considering family blocks.

The ability to estimate per acre yields, hence genetic gain, in mixed family stands remains empirically untested. Nance (1983) simulated the per acre yields of family blocks and mixed family stands of loblolly pine and found that an additive model adequately estimated the yields of the mixed stands. He did, however, uncover several minor inter-family interactions as a result of competition.

In general, the impact of inter-family competition on stand level yield remains largely untested. Studies must be established which relate individual tree level data to stand level yield. Given the general insensitivity of height growth in stands of varying density (Smith 1962), site index for each family in a progeny test may enable accurate simulations of per acre yield at rotation length. In this case, progeny test design may need to be revised to better estimate site index.

CONCLUSIONS

Varying levels of inter-genotypic competition, as displayed in different plot types, appear to have a relatively small effect on family means or ranks; however, estimates of genetic and phenotypic variances and the resultant genetic parameters differ widely by plot type. Family selection for individual tree traits should be effective despite whether the tree breeder uses block plot, row plot, or non-contiguous plot types as long as the progeny test is well designed and implemented. However, selection for per acre yield may depend largely on accurate estimation of site index which may require larger plots or plots with different configurations (e.g., not row plots).

Conversely, genetic and phenotypic variances, family-mean heritabilities, and estimates of realized genetic gain are strongly affected by plot type. Block plots, which involve no inter-family competition, provide family variance estimates that are not confounded with inter-family competition. Not surprisingly the block plot type yields the lowest estimates of family variance and family-mean heritabilities. Accurate estimates of realized gain from family selection for individual tree traits can be derived from block plot progeny tests if the families will also be planted operationally in family blocks. However, if families will be planted operationally in mixtures, the same families must be mixed similarly in trials with appropriate checklots to estimate realized gain. Other progeny test results will provide unrealistic gain estimates. Another approach would utilize growth and yield models to simulate yields from mixed family stands.

TESTABLE HYPOTHESES

1. In pure blocks, families which have been shown to be efficient users of available growing space (e.g., higher volume per unit APA) produce more bole wood volume per acre than families which are inefficient users of available growing space.
2. Families which have been shown to use less than the average growing space and are efficient users of the available space produce more bole wood volume per acre in pure blocks than families which use more than the average growing space and are also relatively inefficient in its use.
3. Binary mixtures, in different ratios, of families are neutral or complementary when both families use less than the average growing space and are efficient users of their available growing space.
4. Binary mixtures, in different ratios, of families are overcompensatory when one family efficiently uses more than the average growing space and the other family efficiently uses less than the average growing space.
5. Binary mixtures, in different ratios, of families are undercompensatory when both families are inefficient in their use of growing space and also use more than the average growing space.
6. Families selected as superior in block plot, row plot and non-contiguous plot tests maintain their relative superiority when compared to a common checklot in: 1) large operational pure block trials and 2) a large operational mixture of the families.
7. There is a large, positive correlation between tree growth and average growing space needed and a large, negative correlation between tree growth and efficient use of available growing space.
8. Data adjustment procedures (e.g., Pinker 1982; Nance et al. 1983; Magnussen 1989) in row or non-contiguous plot trials result in the same ranking of families as in pure block trials.
9. Genetic correlations, before versus after the onset of competition, are larger for block plot trials than either row plot or non-contiguous plot trials.

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GENERAL SESSION:

ADVANCED GENERATION BREEDING STRATEGY

THE NUCLEUS BREEDING SYSTEM

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Abstract. -- A description is given of nucleus breeding as a system where the traditional breeding population is divided into a small elite "nucleus" and a large "main" population. The nucleus population is regenerated each generation by control-pollinations involving assortative mating. This assortative mating, together with intensive combined index selection, is intended to maximise short term genetic gains in the nucleus. These rapid early gains can be transferred into plantations using clonal forestry or seed orchards. Selection and mating in the large main population is less intensive in order to ensure continuing genetic gains in the longer term.

A fundamental feature of nucleus breeding is transfer of genes between main population and nucleus. The nucleus is "open" in the sense that it is replenished each generation by transfer of highly productive breeding individuals from the main population. This transfer from main to nucleus allows genetic improvement made in the main population to be incorporated into the nucleus, thus improving the potential nucleus gain beyond what could be expected in a "closed" structure (with no transfer). Transfer from main to nucleus allows inbreeding in the nucleus to be maintained at acceptable levels.

The transfer of genetic material in the opposite direction, from nucleus to main, allows genetic improvements made by mating outstanding individuals in the nucleus to be incorporated in the main. The paper also discusses investment in tree breeding in terms of optimising the proportion of resources devoted to the nucleus versus the main population.

INTRODUCTION

This paper describes the system of "nucleus breeding" which was first proposed for tree improvement by Cotterill *et al.* (1988). The fundamental feature of nucleus breeding is that breeding effort and investment are concentrated on the best (nucleus) material in the breeding population. The nucleus strategy fits very well with clonal forestry. Nucleus breeding has been a relatively recent innovation in sheep improvement cooperatives in Australia and New Zealand (James 1977, Rae 1979).

CYCLIC IMPROVEMENT

The breeding population is improved each generation by selecting superior trees and then mating these select individuals to produce improved progeny. These progeny are planted in the field to become the next generation breeding population for future selection. Every one (or maybe two) generations the gains made in the breeding population are exploited by taking the very best individuals from the breeding population and using them in seed orchards or clonal forestry (as depicted in Figure 1).

This well known cyclic improvement of the breeding population is the foundation stone of tree breeding. If good gains are not produced from each cycle (generation) of improvement in the breeding population the tree breeding enterprise will eventually fail. The importance of gains in the breeding population is well illustrated by Cotterill and Jackson (1989) in their investigation of alternative seed orchard strategies. These authors demonstrate that over three-quarters of the genetic gain expected from culled second-generation clonal orchards is due to the two cycles of improvement in the breeding population. The other one-quarter of the gain is due to selecting a few outstanding individuals from the second-generation breeding population

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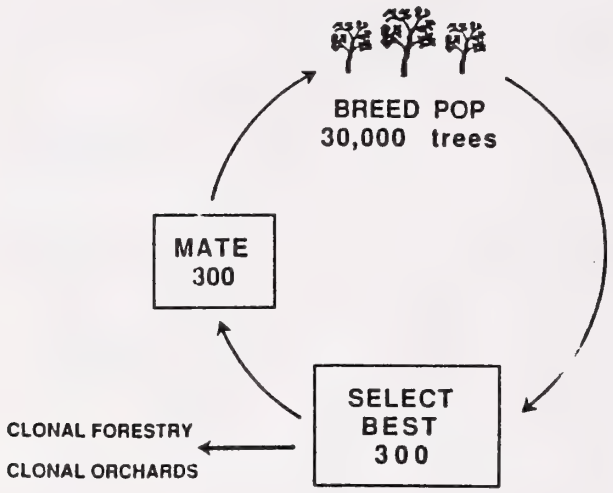
to establish the orchard, a l then subsequently culling the orchard on the basis of progeny test results. Under clonal for try the progress made in the breeding population also has a major influence on the overall su eriority of mass propagated clones.

Figure 1. Cyclic improvement in the breeding population.



POPULATION STRUCTURE

Nucleus breeding involves reorganising the traditional breeding population used in tree improvement to create a two-tier structure including: large "main" breeding population, and elite nucleus population. Following case studies given in Cotterill *et al.* (1988) the main breeding population may be a constant 300 parents each generation which are mated to generate say 30,000 progeny. Cotterill *et al.* (1988) argue that open-pollinated mating may be used to regenerate the main each generation (discussed later).

The nucleus is initiated by screening the main breeding population for outstanding trees. Cotterill *et al.* (1988) suggested that the nucleus population may have around 40 parents each generation which are control-pollinated to produce 10,000 full-sib progeny. However, this population size does not represent a strict rule but merely a guideline for establishing the initial nucleus.

It will become evident that the innovation and fundamental feature of the nucleus system is concentration and mating of elite parents in a distinct and separate population. The nucleus provides a formal population structure for cumulatively capturing gains made from intensive selection and assortative mating from one generation to the next.

TWO-WAY GENE TRANSFER

The nucleus may be "closed" in the sense that transfer of genetic material from main population to nucleus occurs only at the initial screening to establish the nucleus. In subsequent generations there would be no further flow of genes from main to nucleus. The closed nucleus is regenerated after each generation by mating superior individuals selected only from within the nucleus itself.

An "open" nucleus would continue to be replenished each generation by transfer of highly productive breeding individuals from the main population (or from other breeding programs). Outstanding selections from both the nucleus and main would, therefore, be mated to regenerate the nucleus in successive generations. Likewise, there would be a transfer of genetic material in the opposite direction and parents from the nucleus and main are used to regenerate the main.

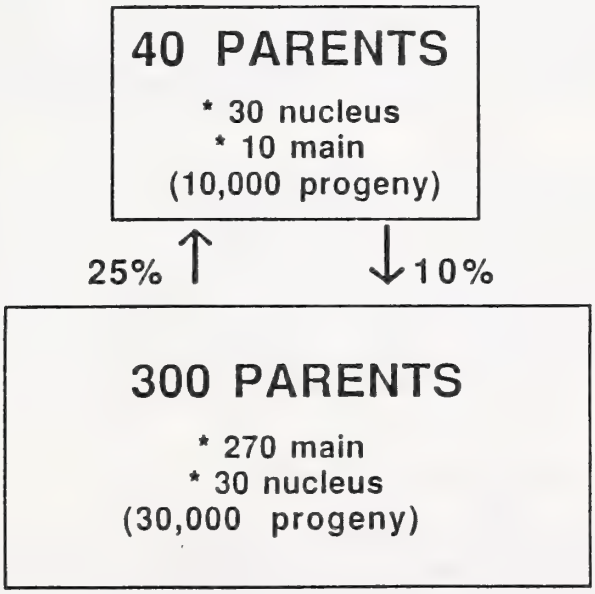
Transfer from Main to Nucleus

Cotterill *et al.* (1988) suggested that the best 30 individuals may be selected (using combined indices) from the 10,000 full-sib nucleus progeny. These 30 individuals, together with 10 of the best new generation selections from the main population, are control-crossed in 100 combinations to produce 10,000 progeny to regenerate the nucleus population (Figure 2). Of the 40 parents used to regenerate the nucleus each generation, 25% (or 10 parents out of 40) are therefore selected in the main population and the remaining 75% (30 parents) are selected in the nucleus. Research into nucleus breeding in animals and trees suggests that when 25 to 50% of the nucleus parents are selected in the main the rate of increase of inbreeding in the nucleus is minimised and longer-term gains are maximised (James 1977, 1978, Cotterill 1990).

The 10 selections from the main population can be transferred to the nucleus as pollen and consequently serve as male parents in the control-crossing. Cotterill (1990) showed how this procedure can lead to the same generation time in the nucleus as in the main population.

This transfer of genetic material from main to nucleus allows genetic improvement made in the main population to be incorporated into the nucleus, thus improving the potential nucleus gains beyond what could be expected in a closed population (James 1977). As already mentioned, transfer from main to nucleus may also allow inbreeding in the nucleus to be maintained at acceptable levels over successive generations (James 1978).

Figure 2. Main and nucleus population structure with two-way transfer of genes.



Transfer from Nucleus to Main

Following the case studies given in Cotterill *et al.* (1988) the 30 individuals selected in the nucleus population are used as parents in the main as well as the nucleus. Of the 300 female parents used to regenerate the main, 10% (or 30 parents) are therefore selected in the nucleus population and the remaining 90% (or 270 parents) are selected in the main (Figure 2). This transfer of genetic material from nucleus to main allows genetic improvement made in the nucleus to be incorporated into the main. We will see later that the genetic gains expected in the main population are enhanced by this scheme.

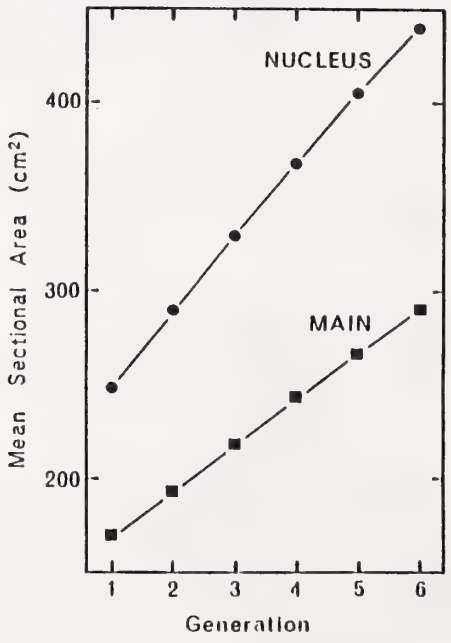
The open nucleus approach with two-way transfer of genetic material represents a fundamental shift away from the strictly one-way flow of genes from bottom to top of the hierarchy of populations (i.e. from breeding population to seed orchards) which is a feature of traditional tree improvement. The seed orchards have been "dead ends" and contributed only to present progress but not to continuing progress. This is because the genetic changes made by mating outstanding parents in orchards are not transferred back into the breeding population.

EXPECTED GENETIC GAINS

Gain Calculations

Figure 3 presents genetic gains expected from selection on sectional area of stem over six generations in the nucleus and main populations. These gain calculations are taken from case studies for *Pinus radiata* in Australia and *Eucalyptus globulus* in Portugal (as taken from Cotterill *et al.* 1988). The sectional area of stem is assumed to be measured at six to eight years after planting and have an individual heritability around $h^2=0.2$ for both species. The main population is assumed to be regenerated by open-pollinated mating each generation and the nucleus by control-crossing (Cotterill *et al.* 1988). Theory for determining expected gains under nucleus breeding in forestry is presented in Cotterill (1990).

Figure 3. Cumulative gains expected in sectional area in the nucleus and main populations over six generations of selection.



It is apparent from Figure 3 that the gains expected per generation in the nucleus far exceed corresponding gains expected in the main population. Having provided the most productive parents to initially establish the nucleus an "improvement lag" develops between the nucleus and main populations. The rapid early gains in the nucleus lead to a doubling of this improvement lag over the first six generations. Early financial returns from rapid progress in the nucleus and widening of the improvement lag has been a potent factor in the acceptance of nucleus breeding in the sheep industry (Rae 1979).

If the system is continued as recurrent cycles of selection and transfer (in both directions) the gain in the nucleus will diminish and the gain in the main increase. The main population will eventually make the same rate of genetic progress as the nucleus and the improvement lag becomes constant (James 1977, Rae 1979). However, it may take many generations before this point of stability is reached and it could therefore be of little practical importance in tree breeding.

The gains in sectional area after the first-generation in Figure 3 represent a 14% change in mean of the main population and a 24% change in mean of the nucleus. It is interesting to note that without transfer of genetic material from nucleus to main the expected gain in the main would have been only 11% per generation (Cotterill *et al.* 1988). The transfer from nucleus to main is therefore important in enhancing progress in the main.

Nucleus Gains

The rapid gains in the nucleus population (Figure 3) are due to three factors:

1. Selection Intensity. High selection intensities are used in choosing the 30 nucleus parents out of 10,000 trees within the nucleus itself and, in particular, in choosing the remaining 10 nucleus parents out of 30,000 trees in the main population.

2. Family Information. The combined indices used to select nucleus parents can place far greater emphasis on family information compared with combined indices used to select main parents. This is because the nucleus is open and any build-up in inbreeding will be alleviated by transfer of unrelated (or less related) pollen from the main population. However, in the main population it is essential to avoid rapid increases in inbreeding as a consequence of placing heavy emphasis on family information in combined indices.

3. Assortative Mating. The control-pollinations in the nucleus represent a form of assortative mating of best parents with best parents. These pollinations will not follow a rigid mating pattern such as diallels or factorials. Rather than follow a predetermined pattern, "best mate" indices are used to attempt to predict which of the 40^2 crossing combinations among the 40 nucleus parents should produce the most economically valuable progeny. The 100 combinations which appear to be potentially most valuable would subsequently be completed in the control-pollination program. The potential advantages of assortative mating are highlighted by Cotterill *et al.* (1987), and details of constructing best mate indices are given in Cotterill and Dean (1989).

EXPLOITING NUCLEUS MATERIAL

There is no doubt that nucleus breeding fits neatly with clonal forestry. The nucleus acts as a "packaging" population where desirable features of a few outstanding parents are wrapped together in full-sib families. The most highly productive families (or individuals) can be quickly mass vegetatively propagated into plantations. However, nucleus breeding also fits with the more traditional orchard strategies. It is possible to create clonal seed orchards using best unrelated (or least related) material from the nucleus. Alternatively the nucleus population on some sites can be converted into seedling orchards.

REDIRECTING INVESTMENT

It is easily argued that investment in tree improvement should be concentrated on those parts of the breeding program which will give greatest economic returns. Some breeding programs devote large proportions of resources to completing control-pollinations among large numbers of parents in the (main) breeding population. For instance, *P. radiata* breeding operations in Australia have invested up to 40% of their total labour and financial resources in completing extensive control-pollination programs to regenerate big populations.

Cotterill *et al.* (1988) suggested that the main breeding population may be regenerated quickly and at low cost using open-pollinated mating. The idea is that resources saved by implementing open-pollination in the main population can be redirected to pay for intensive control-pollinations in the nucleus. In this way the nucleus provides a focus for financial investment. However, the nucleus system is not dependent on using any particular mating pattern in either the nucleus or main.

ADVANTAGES OF NUCLEUS BREEDING

Genetic Gains

The potential gains in the nucleus population far exceed those which could be expected from traditional strategies of cumulatively improving a single large breeding population. This is because the best breeding individuals are concentrated in the nucleus and bred together under assortative mating. The early gains in the nucleus are sustained by intensive selection in the nucleus itself and continued replenishment from highly productive individuals from the main population (and other breeding programs).

Economic Success

The financial investment in traditional tree breeding has often been spread thinly in improving a large, relatively slow moving, breeding population by extensive control-crossing and testing. Under nucleus breeding the investment and breeding effort can be focussed on the smaller nucleus population containing outstanding genetic material. This focussing of investment on a population which is expected to yield rapid gains should ensure good financial success of nucleus breeding. The need to make a commercial success of the breeding enterprise is clearly a critical consideration.

Versatility

The nucleus is a very versatile population in the sense that it is ideal for introducing and testing the best selections from outside breeding programs. These outside introductions may even be from different species and the nucleus would therefore include hybrid material. The nucleus would also be an ideal population for gene engineering research and development.

Breeding Cooperatives

The nucleus strategy may provide a convenient structure for tree improvement cooperatives (as it has for sheep cooperatives). Each partner in the cooperative could have their own nucleus population and be responsible for crossing and testing this commercially valuable material. However, the cost of carrying the large main population necessary to replenish each nucleus could be shared amongst cooperators. Alternatively, the reverse may apply with the nucleus being run by the cooperative and each partner having their own main population contributing to the central nucleus.

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MODELING ELITE POPULATIONS AND POSITIVE ASSORTATIVE MATING IN RECURRENT SELECTION PROGRAMS FOR GENERAL COMBINING ABILITY

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Abstract.-- An algorithm for allele modeling demonstrated the efficacy of adding an elite, highly selected population to the hierarchy of populations currently employed in tree breeding programs. Reselection in a large main-line breeding population (N=498) to establish an elite breeding population (n=48) gave an immediate increase in genetic gains that was not offset by inbreeding depression and declining additive genetic variance until after 10 generations of breeding and selection. Positive assortative mating within the elite population yielded a small additional increment of genetic gain. Positive assortative mating did not significantly increase the proportion of extreme genotypes that might be used in vegetative propagation programs in the presence of selection.

Keywords: elite populations, positive assortative mating, computer simulation, enrichment.

INTRODUCTION

Tree breeders continually seek ways to increase the genetic gains that are realized from current tree breeding programs. If vegetative propagation was practical, specific combining ability (sca) could be capitalized upon to increase genetic gains in addition to the general combining ability (gca) upon which most current programs are based. But methods to vegetatively propagate the southern pines economically are still being sought. Genetic gains could also be increased by employing breeding strategies that would advance sca in breeding populations. However, questions about the levels and persistence of sca effects over time (Byram and Lowe 1986), and the requirement for multiple breeding populations (McKeand et al. 1986), are the major reasons that breeding strategies have not been adopted that would advance both sca and gca.

Positive assortative mating (PAM), coupled with vegetative propagation, has been proposed as a method to increase genetic gains from traditional breeding programs (Foster 1986). In theory, more gain can be achieved with PAM by increasing the component of additive genetic variance available for selection (by generating gametic phase disequilibrium) and by producing good specific crosses at a higher frequency than by present methods, where good specific combinations only occur at random. Assortative mating is the mating of individuals with the same phenotype more often than would occur by chance.

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It is positive when the phenotypic value of both parents deviates from the population mean in the same direction (Gianola 1982). That is, PAM mates the first-ranked parent to the second-ranked, third-ranked to fourth-ranked, etc., until the next-to-last and the last ranked are mated.

In practice, breeders are likely to cull, not mate, the poorest individuals. Thus, PAM would be practiced on a selected subset of the breeding population. In such a scheme, genetic gains would arise not only from PAM, but also from reselection to form a smaller, elite population. Relatedness would be expected to increase more rapidly in smaller, elite populations than in the larger, main-line population from which they were drawn. Thus, inbreeding depression would eventually offset genetic gains and necessitate enriching the smaller population from the larger.

In this study, elite populations were considered as an additional level in the hierarchy of populations currently employed in tree improvement programs. PAM could practically be employed in elite populations to enhance gains from reselection.

Specifically, this study was undertaken to: (1) evaluate the use of elite populations in an hierarchical breeding strategy, (2) evaluate the use of PAM in elite populations, (3) estimate the impact of inbreeding depression within elite populations of two sizes, and (4) evaluate the effectiveness of enrichment from a larger main-line population.

METHODS

The computer simulation used FORTRAN 77 to model one quantitative trait with 50 loci. Every locus had partial dominance gene action with a degree of dominance of one-half, i.e. AA genotype=2.0, Aa genotype=1.5, and aa genotype=0.0. Dominance variance was approximately one-seventh of the total genetic variation. There were two alleles per locus of equal frequency at time zero that were permitted to recombine freely, i.e., there was no linkage. Two elite population sizes, n=48 and n=12, were reselected from a large (N=498), main-line population. Heritabilities of 0.1, 0.2, and 0.3 were modeled. Only the results with the highest heritability (0.3) are reported here.

Increases in the inbreeding coefficient result when relatives are mated. In the presence of dominance variance, increased inbreeding results in inbreeding depression in cross-bred species. Phenotypic means of offspring populations were reduced to account for inbreeding depression. Based on the reduction in growth of Pinus radiata D. Don (Griffin et al. 1986) and Pinus elliottii Engelm. (Gansel 1971) with increasing inbreeding coefficients, a 5% reduction in growth was made for every 0.1 increase in F-value.

All genotypes were generated in the first generation in the main-line breeding population, which was modeled to approximate current tree improvement practices (Figure 1). It was assumed that it would take twice as long to complete a breeding cycle in the large, main-line population as in the small, elite population. Thus, the main-line and elite populations were modeled for

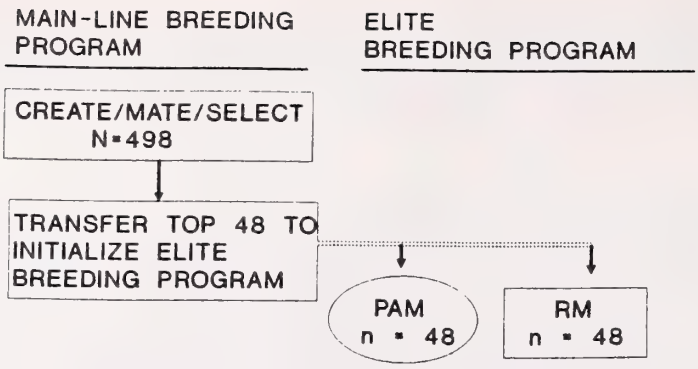
MAIN-LINE BREEDING PROGRAM
ELITE BREEDING PROGRAM
CREATE/MATE/SELECT N=498
TRANSFER TOP 48 TO INITIALIZE ELITE BREEDING PROGRAM
PAM n = 48
RM n = 48

Figure 1. Initiation of genotypes in the hierarchy of breeding populations modeled.

11 and 21 generations, respectively. Results were based on averages of twenty replicate runs for each combination of factors modeled. Realized gains were the differences between the phenotypic means of successive progeny populations. The units of gain were arbitrary, i.e. an individual's phenotypic value was the sum of genotypic values over loci plus a randomly-assigned environmental deviate.

The best genotypes were copied from the main-line population after one breeding cycle was completed to create identical initial populations for two elite populations in generation one. One elite population was mated using positive assortative mating (the PAM population) while the second was randomly mated (the RM population, Figure 1). Thus, differences in cumulative gains, adjusted for inbreeding depression, between the RM and main-line populations were due to reselection and establishing a new level in the breeding population hierarchy, while differences between the PAM and RM elite populations were due to positive assortative mating.

All three population sizes were maintained over generations. Family plus within-family selection was employed with the restrictions that only one individual was selected from a full-sib family; and no half-sib parent was represented more than three times in the selected population. Random or positive assortative mating was done using six-parent disconnected half diallels.

RESULTS AND DISCUSSION

The incremental gain from reselection to establish elite populations is shown in Figure 2A. For the elite populations of size 48, gains for the RM population were 4% to 45% greater than for the main-line population for 12 generations, while the PAM population was 1% to 40% greater for 14 generations (Table 1). The increase in gain from positive assortative mating (PAM versus RM) varied between 0%-6% through 14 generations of mating. The percentage gains declined because the base for their calculation, cumulative adjusted gains in the main-line population, increased over time. The absolute value for incremental gains from reselection were maintained for 10 generations of mating and selection in the elite populations.

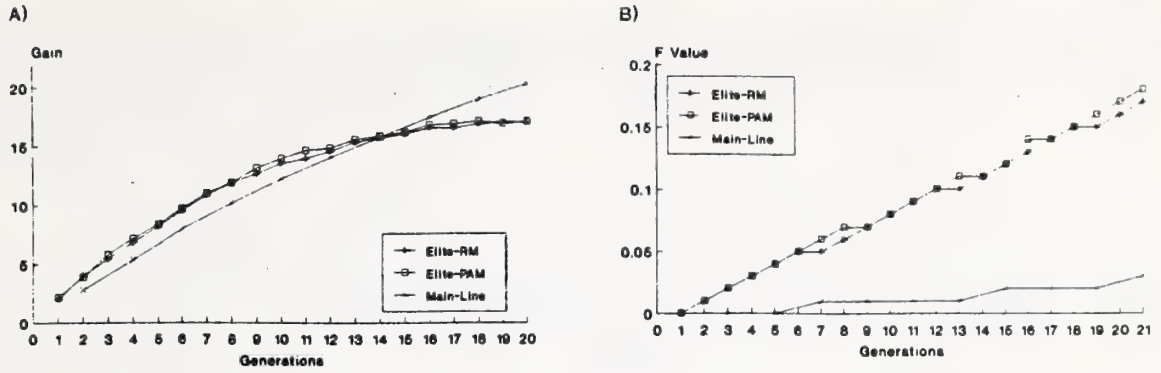

Figure 2. Adjusted cumulative gain (A) and inbreeding coefficient (B) for elite population size 48 and h^2=0.3.

Table 1. Percentage change in adjusted cumulative gain for elite populations over the main-line population.

Generation	Elite-PAM(%)	Elite-RM(%)
2	40	45
4	34	28
6	21	20
8	17	17
10	14	11
12	6	4
14	1	-.5
16	-4	- 5
18	-10	-11
20	-16	-16

Closed breeding in the elite population led to increased inbreeding. Inbreeding coefficients reached 0.16 to 0.18 by generation 21 for the n=48 populations (Figure 2B). The penalty for inbreeding depression and reductions in the additive genetic variance (Figure 3) began to offset gains in the elite population after generation 10. Additive genetic variance declined as the result of selection.

The small (n=12) breeding population reached an inbreeding coefficient of 0.15 in generation four (Figure 4B), in contrast to generation 19 for n=48 (Figure 2B). Adjusted cumulative gain fell below that for the main-line by the third generation for both PAM and RM populations (Figure 4A). Enrichment would thus have been required at generation two to maintain gains above those possible in the main-line population. Done on this schedule, enrichment would

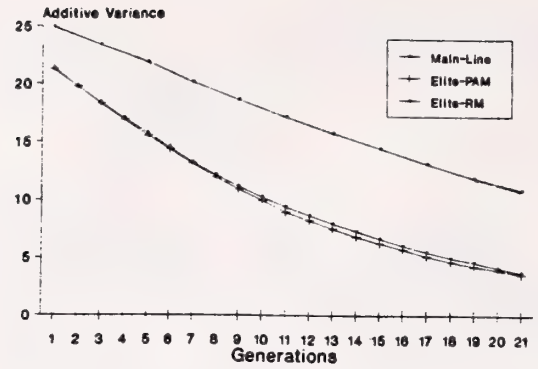

Figure 3. Additive genetic variance for the main-line population and elite populations of size 48, h^2=0.3.

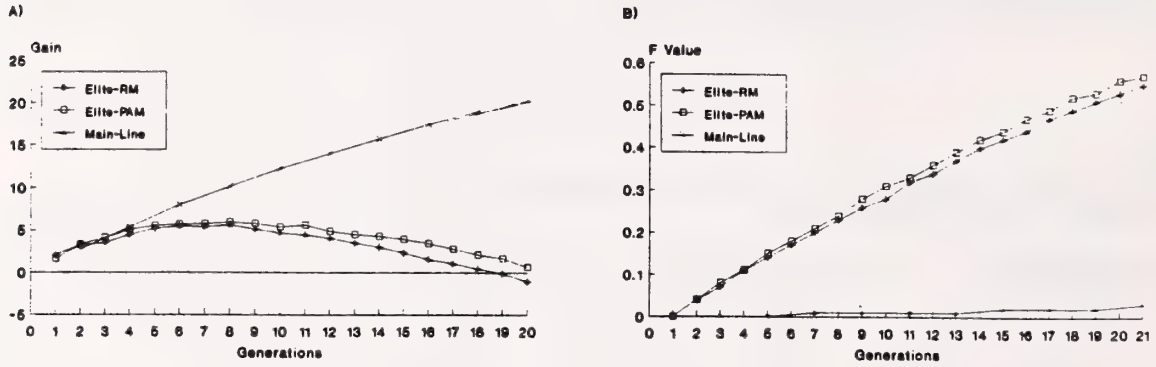

Figure 4. Adjusted cumulative gain (A) and inbreeding coefficient (B) for elite population size 12 and h^2=0.3.

simply be sampling another small population from the main-line population, and gains would again quickly decline again with closed-population breeding. Thus, periodic enrichment to offset increases in inbreeding and restore additive genetic variance was evaluated only for elite populations of size 48.

The enrichment methodology employed began in generation two. Each time a breeding cycle was completed in the main-line population, the top 24 individuals from the main-line population replaced the poorer half of the selected individuals in the elite populations. This enrichment strategy was chosen because at least 50 phenotypes in the main-line population exceeded the mean of the selected offspring in the elite population. No restrictions were placed on the pedigrees of the transferred phenotypes. It was assumed that the level of coancestry among individuals transferred from the main-line would be small in a large, randomly-mated population.

Enrichment reduced the F-value of the elite populations from their previous levels of 0.16 and 0.18 in generation 21 to 0.03; and additive genetic variance increased moderately in the elite populations with

enrichment. However, cumulative genetic gains in the elite populations were not greater than for the elite populations without enrichment. This occurred because selection was imperfect at the low heritability used (0.3), and the replacement genotypes were no better, on average, than the genotypes they replaced in the elite populations.

Even though simulation of this strategy showed that elite populations of size 48 would best be handled as closed breeding populations, enrichment may still be a viable alternative. If replacement genotypes were more accurately estimated, perhaps by progeny testing, and less than 50% of the of the elite population were replaced, gains might be increased or at least maintained in elite populations.

The increase in the numbers of extreme individuals produced with PAM was small. The number of phenotypes greater than two standard deviations above the mean of the elite populations (n=48) varied from two to six per breeding cycle. This agreed with the findings of Breese (1956), who demonstrated that the increase in extreme genotypes was not large when the number of loci controlling a trait was large and heritability was not one. Also, the present model combined selection with PAM. Thus, the poorer phenotypes were discarded and the population variance decreased, resulting in fewer extreme phenotypes.

CONCLUSIONS

Establishing an elite, closed breeding population with 48 individuals selected from a large, main-line breeding population yielded more gain than the large main-line population until generation 14. Positive assortative mating in the elite population added a small, additional increment of gain, and slightly increased the numbers of extremely good individuals.

Inbreeding depression and reduced additive genetic variance in a smaller (n=12) elite population reduced gains from reselection below that for the main-line by generation three. With n=48, gains from reselection did not begin to decline relative to those in the main-line population until generation 10.

The one enrichment strategy modeled demonstrated that it may be necessary to progeny test genotypes before transfer to elite populations, and/or replace fewer than one-half of the selected individuals in the elite population.

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RESTRICTION FRAGMENT LENGTH POLYMORPHISMS AND THEIR POTENTIAL USE IN MARKER ASSISTED SELECTION IN SOUTHERN PINE IMPROVEMENT

Warren L. Nance and C. Dana Nelson[1]

Abstract.--A new class of molecular markers based on restriction fragment length polymorphisms (RFLPs) is described. The use of RFLPs in exposing the genetic basis of phenotypic variation is then explored from a tree breeders perspective. It is concluded that RFLP markers developed in conjunction with multi-generation pedigrees and properly designed experiments will eventually make it possible to apply marker assisted selection to southern pine improvement programs.

Keywords: restriction fragment length polymorphisms (RFLPs), linkage, fusiform rust resistance, marker assisted selection, gene mapping.

INTRODUCTION

Genetic markers by definition are monogenically inherited traits with heritability equal to one. Quantitative geneticists, using linkage concepts, have developed a body of theory that attempts to utilize the perfect heritability of genetic markers to increase the efficiency of selection for traits of low heritability (Jayacker 1970, Mather and Jinks 1971, Thoday 1961). This theory, termed marker assisted selection, seems especially appropriate for use in forest tree improvement, since the long generation interval and large size of forest trees cause extensive progeny testing to be very expensive. Many methods and applications of this approach have been described and tested with agronomic crops and farm animal species (Soller 1978, Soller and Beckmann 1983, Soller and Plotkin-Hazan 1977, Stuber et al. 1982, Tanksley et al. 1981, Tanksley and Rick 1980.

Most markers traditionally studied by geneticists have been either morphological or molecular in nature (Tanksley 1983). Forest geneticists have traditionally identified and studied molecular markers -- primarily monoterpene and enzyme variants (Conkle 1981, Squillace et al. 1980). Most applications of these molecular markers in forest trees have been aimed at understanding the genetic structure and mating systems of both natural and artificial populations (El-Kassaby and Ritland 1986, Knowles 1985, Neale and Adams 1985). Although monoterpenes and isoenzymes have proven to be very useful in specific applications, they are not expected to be generally useful in marker assisted selection efforts due mainly to their limited number and lower level of polymorphism (Beckmann and Soller 1983). Limited numbers of markers decrease the probabilities of finding markers closely linked to important loci, and low levels of polymorphism decrease the probability of finding informative pedigrees.

Within the last few years, a new class of molecular markers, restriction fragment length polymorphisms (RFLPs), has been developed which should overcome the limitations of the present marker systems (Botstein et al. 1980). RFLPs are expected and in many cases have been shown to have the following desirable features: (1) almost limitless in number, (2) highly variable or polymorphic, (3) co-dominant expression, and (4) lack of pleiotropic effects on other characters (Beckmann and Soller 1983, Tanksley 1983). RFLPs have recently been discovered in the chloroplast DNA of gymnosperms (Neale et al. 1986, Wagner et al. 1987) and there is hope that RFLPs will soon be available for nuclear and mitochondrial DNA in the southern pines.

The purpose of this paper is twofold: (1) to describe in detail RFLPs and RFLP loci and (2) to begin exploring the use of RFLP loci in forest genetic experiments and tree improvement programs.

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RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

The fact that deoxyribonucleic acid (DNA) is the informational molecule that encodes fundamental life processes was only convincingly demonstrated in 1944 (Avery et al. 1944). Nine years later the precise structure of DNA was discovered (Watson and Crick 1953), and eleven more years passed before the complete genetic code was finally deciphered (Anon. 1966). Despite these remarkable advances, detailed studies of specific regions of the DNA molecule itself proved impossible. Then, in 1970 a new class of enzymes called restriction endonucleases was discovered that allowed sequence-specific assays of the DNA molecule (Smith and Wilcox 1970). With the aid of these enzymes, greatly accelerated progress in many areas of molecular genetics has been made in the last two decades (Watson et al. 1987).

Restriction Enzymes

An understanding of restriction enzymes and their relationship to RFLPs requires some knowledge of molecular genetics, including the structure of DNA and various chemicals and enzymes, and laboratory procedures related to the manipulation of DNA. Watson et al. (1987) provide an excellent overview of the subject at a more advanced level, while Drlica (1984) provides a more general introduction to the subject.

Restriction enzymes are generally extracted from bacterial cells, where they provide a defense mechanism against the functioning of foreign DNA. The restriction enzymes bind only to specific base sequences (usually 4 to 8 bases long) in the foreign DNA and subsequently cut both strands of DNA at the recognition (restriction) site, thus fragmenting the invading DNA and generally destroying its function. The microorganism's DNA is protected from this fragmentation by the chemical modification (methylation) of its own recognition sites, which effectively blocks the action of the enzymes.

Figure 1 illustrates the cleavage of DNA by a particular restriction enzyme, MstII. The MstII restriction enzyme cleaves DNA within the recognition site CCTGAGG between the second and third base from the 5' end, leaving three unpaired nucleotides at the 5' ends. These so-called "sticky ends" allow the insertion of any similarly restricted (MstII) double-stranded DNA

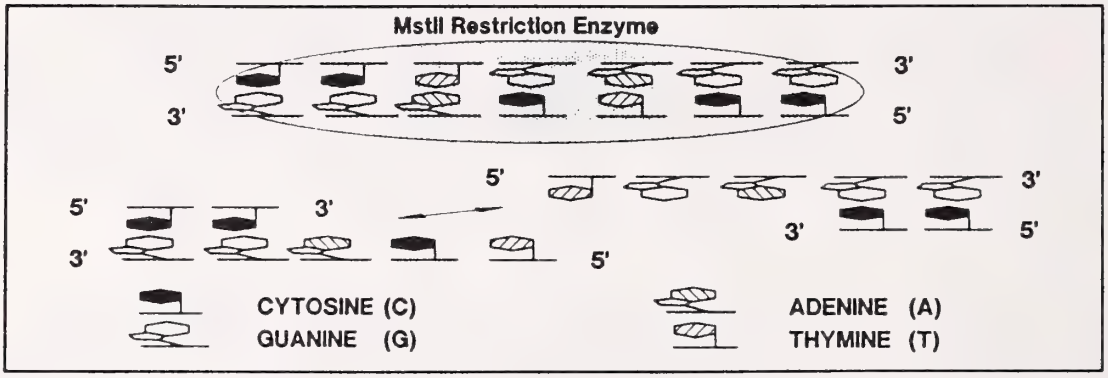

Figure 1. Restriction enzyme MstII recognition site and cleavage of DNA.

fragment into the gap created by the restriction. The insertion of foreign DNA in this way produces a "recombined" or "genetically engineered" DNA molecule. This application of restriction enzymes is one of their primary uses, and without restriction enzymes recombinant DNA technology would not be possible.

However, restriction enzymes are not limited to recombinant DNA work, where the major goal is to manipulate and restructure genomes. Perhaps an even more important and immediate application for these enzymes is to help geneticists expose variation between individuals at the

most fundamental level, the DNA molecule itself (Beckmann and Soller 1986). This level of genetic variation, DNA polymorphism, need not be directly involved in phenotypic variation to be useful to geneticists and breeders. For example, many regions of DNA in eucaryotic genomes are not part of functional genes nor are they involved in the regulation or expression of functional genes (Watson et al. 1987). Yet many of the most productive applications of DNA polymorphisms (including molecular marker systems, genetic fingerprinting, and phylogenetics) are actually enhanced by the absence of selection pressure and/or pleiotropic effects that is characteristic of DNA polymorphisms from non-functional DNA regions.

Restriction Enzyme Analysis of DNA Polymorphisms

The technology and procedures used to expose DNA polymorphisms rely on the unique power of restriction enzymes to fragment DNA in a sequence-specific manner. Restriction enzymes are used to cut the DNA molecule under study into fragments, and the number and lengths of those fragments (the fragment length array) depend on the number and distribution of the particular enzyme's recognition sites. The fundamental idea is that two DNA molecules are considered to be polymorphic with respect to one another if they produce different fragment length arrays when cut with the same enzyme. Such an enzyme is said to have exposed a DNA polymorphism which is observed as a restriction fragment length polymorphism (RFLP).

Two common sources of variation generally account for RFLPs (White and Lalouel 1988): (1) two DNA molecules may differ in the number of restriction sites cut by a particular enzyme, and/or (2) two molecules may differ in the length of DNA sequences separating common restriction sites. The former may arise as a result of point mutations that either destroy a restriction site or create a new restriction site. The latter may arise as a result of variable numbers of repeating sequences (VNR) occurring between restriction sites.

For small haploid genomes (such as DNA from most viruses and some bacteria), the array of DNA fragments produced by a restriction enzyme digest may consist of only a few fragments (perhaps less than 25 or so), and in such cases the fragment array can often be sorted by length and directly visualized using agarose gel electrophoresis (Maniatis et al. 1982). When the gel is stained with a DNA-specific stain such as ethidium bromide and exposed to ultraviolet light, the DNA fluoresces in the visible spectrum. The pattern of electrophoretic bands produced by the digestion of a genome with one or more restriction enzymes is a karyotype (a restriction fragment length karyotype), and a comparison of such karyotypes from different individuals or populations can provide evidence of RFLPs. The procedure is illustrated in figure 2.

For large genomes, such as the nuclear (diploid) genome of humans and the even larger genome of gymnosperms, the individual fragments from a restriction digest cannot be visualized in the gel as separate bands due to the large number of fragments produced and their nearly continuous size variation. When the gel is stained and illuminated, a smear of DNA, rather than separate bands, is produced. Fortunately, a procedure known as Southern blotting (Southern 1975) can be used to expose restriction fragment length variation in DNA smears. The basic idea of this procedure is to allow hybridization (double-strand formation) between a single-stranded, P^{32}-labeled DNA clone (probe) and single-stranded, electrophoresed (target) DNA. The hybridization occurs only between homologous (complementary) base sequences and its result is observable with the aid of radioactivity and X-ray film.

Figure 3 illustrates the Southern blotting procedure which includes the following steps: (1) digest target DNA with a restriction enzyme, (2) size-separate digested DNA in gel electrophoresis, (3) denature (split into single-strands) size-separated DNA fragments, (4) transfer (by capillary action) single-stranded DNA fragments to a rigid support (e.g. nitrocellulose or nylon) membrane, (5) bind DNA fragments to the membrane with ultraviolet light or heat, (6) place membrane into a hybridization solution and add multiple copies of a P^{32}-labeled, single-stranded probe DNA, (7) allow hybridization reaction to occur between probe and target DNA, (8) wash membrane of excess, unbound probe DNA and expose to autoradiographic film, (9) develop film and observe bands. The observed bands reveal the length of the target DNA fragments that were homologous to the probe DNA.

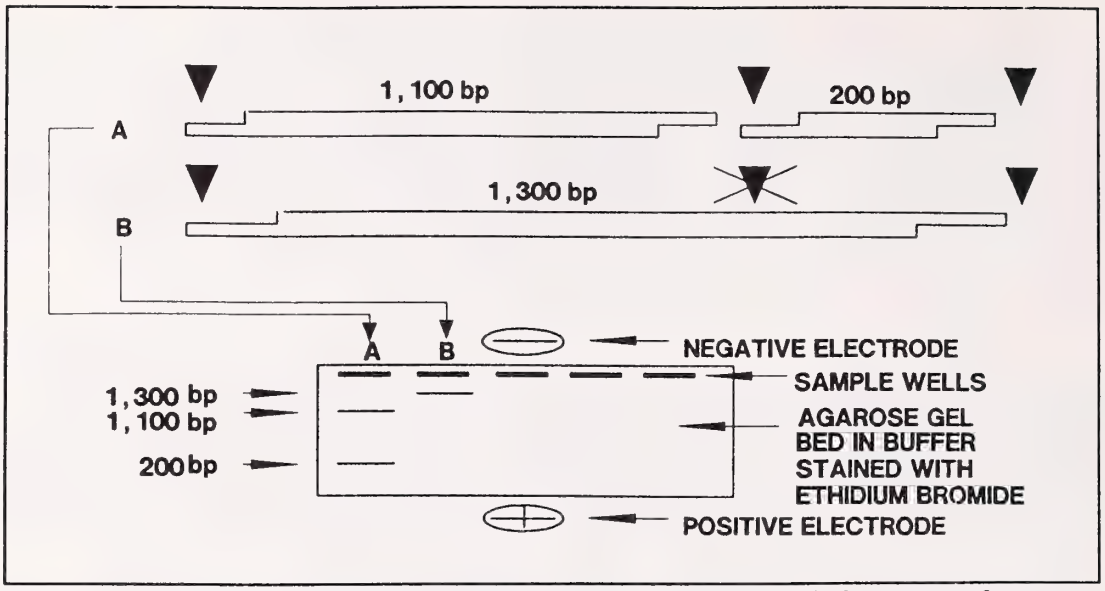

Figure 2. Restriction digest and electrophoretic separation of fragments from two individuals A and B. B is missing an MstII restriction site in this 1,300 base pair region.

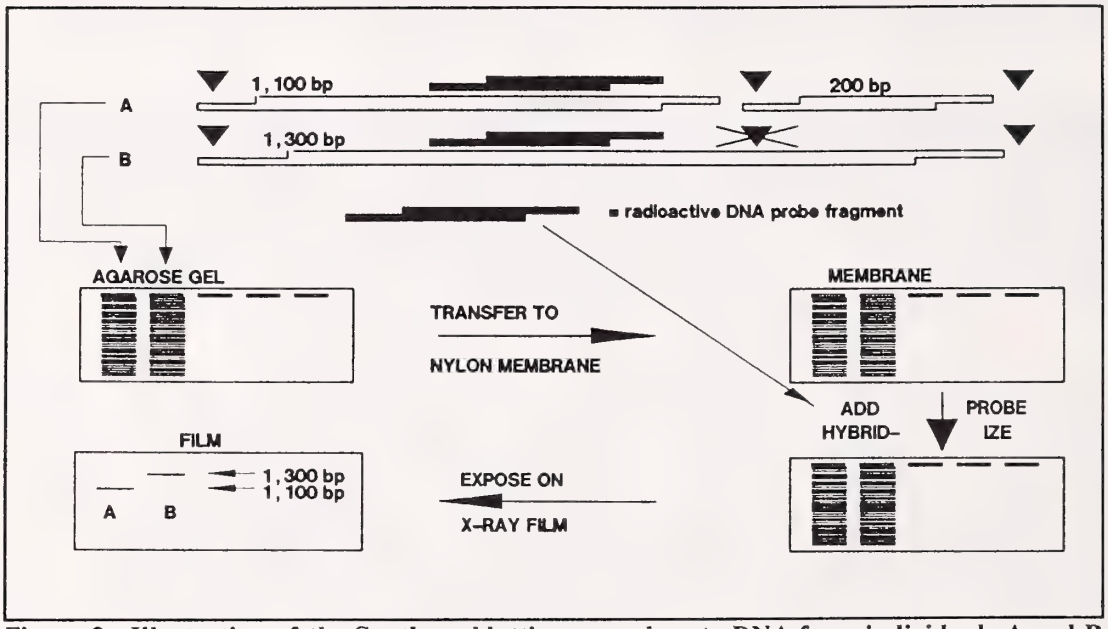

Figure 3. Illustration of the Southern blotting procedure to DNA from individuals A and B in figure 2. See text for description of each step.

Probe Libraries

The choice of a probe to use in Southern blotting is, of course, critical to the success of the procedure. To be useful, a probe must be homologous to the target DNA fragment(s) that expose polymorphisms. To assure homology, probes may be generated by randomly fragmenting DNA from the same genome (or a closely related one) under study. Using recombinant DNA technology, the fragments are inserted into a vector (such as a plasmid, virus, or a cosmid)

capable of preserving and reproducing (i.e. cloning) the inserted DNA fragments. Cloned fragments then must be examined to detect and eliminate those with highly repetitive sequences. This kind of probe library is called a unique-sequence random genomic library, and such libraries have proven to be useful in detecting RFLPs in many species (Helentjaris et al. 1986, Helentjaris and Gesteland 1983, White and Lalouel 1988).

Another type of library, called a cDNA library, is constructed by first extracting messenger RNA and then using an enzyme (reverse transcriptase) to produce a complementary DNA (cDNA) copy of each of the various RNA molecules extracted. The assortment of cDNA molecules produced in this way can each be inserted into a vector and handled in the same way that random genomic probes are handled. Because the cDNA probes are homologous to actively transcribed regions of DNA in the genome, they are homologous to functional regions of the genome and are potentially more useful, though more limited in number, than random probes.

A useful probe library (whether unique-sequence random genomic or cDNA) will contain many probes that are homologous to the target genome, but only a small percentage may be useful in exposing restriction fragment length variation. Each probe/enzyme combination has the potential of producing a different banding pattern, and many different probe/enzyme combinations may have to be screened before a suitable combination is discovered which exposes polymorphisms for the DNA samples under study. Fortunately, membranes can be reprobed many times (20 or more) and probes may also be multiplexed (pooled) in one hybridization experiment to allow the convenient screening of many probe/enzyme combinations. Given the growing number of restriction enzymes (>200) commercially available and hundreds of probes that can be generated and maintained in libraries, the possible enzyme/probe combinations that could be screened are enormous. Short of completely sequencing the DNA, no other system can provide such a rich reservoir of potential polymorphic markers.

RFLP Loci and Linkage

For haploid genomes, a given enzyme/probe combination which exposes a single band of equivalent length for each haploid individual analyzed is essentially equivalent to a genetic locus with a single allele. Such loci are not polymorphic in that one cannot distinguish individuals one from the other in the population based on these loci. On the other hand, if at least one sample exhibits a single band of different length than the others, then there is more than one allele at this locus and there is evidence of DNA polymorphisms in the population.

For diploid individuals, a unique-sequence probe that hybridizes to and exposes a single band on each homologous chromosome will produce only one band for homozygous individuals. The position of that band defines the allele carried by that homozygote, and the locus is defined by the particular enzyme/probe combination used. A cross between two homozygotes carrying different alleles for the locus is expected to produce heterozygous offspring with two bands, the position of the bands should correspond to the positions observed in the parents. If the inheritance pattern in the offspring is as expected, then the existence of the RFLP locus is confirmed, otherwise an anomaly discounts the locus. It is thus essential to confirm RFLP loci with inheritance data before accepting such loci as markers.

Upon finding RFLP loci, it is vital to begin arranging these loci into linkage groups. Loci whose alleles freely recombine during meiosis are unlinked and belong to different linkage groups, whereas those that do not freely recombine belong to the same linkage group. A direct and several indirect methods are available for linkage analysis (Ott 1985). The direct method relies on counting recombinants and non-recombinants, taking the proportion of recombinants as theta, the recombination fraction. Theta varies from 0, for complete linkage (i.e. same loci) to .5, for no linkage. In practice the direct method can only be applied under ideal conditions to organisms where inbred lines are available.

The indirect method of likelihood and LOD score appears to be of greater utility in studying linkage in forest tree pedigrees. The likelihood is the probability that the observed data occur under a specified model. In the linkage analysis case, the observations are genotypes, two

alleles present at each locus, and the model consists of only one parameter, theta. The object of the linkage (two point) analysis for a given pedigree and pair of loci is then to determine the likelihood of the observations over the range of possible theta (0 to .5), typically .01, .05, .1, .2, .3, and .4.

To accept a linkage hypothesis, a likelihood or odds ratio of 1000 to 1 is required, where the odds ratio is the probability of the observations at a specified theta (<.5) over their probability at theta =.5 (no linkage). An LOD score, obtained by taking the log (base 10) of the odds ratio, of 3 is equivalent to an odds ratio of 1000 to 1 and "proves" linkage. Alternatively, a linkage hypothesis is rejected when an LOD score of -2 (1 to 100 odds ratio) is reached. LOD scores for pairs of loci are computed for each unrelated pedigree and added to produce composite (current) LOD scores, one composite score for each value of theta. If an LOD score of 3 is reached for any theta, then that value which maximizes the LOD score is taken as the maximum likelihood estimate of theta.

Estimates of theta between all known loci allow the relative arrangement or map of these loci. The extension of the above two point analysis concepts to multi-point analysis greatly increases the precision of the order and theta estimates among all known loci (Lander and Green 1987, Lathrop and Lalouel 1984). The result is a likelihood for each possible or hypothesized order of loci and estimates of theta between each loci in the given order (Lander et al. 1987). Thus, likelihoods of alternative maps may be compared, analogously to comparing likelihoods of alternative theta. A strategy for mapping genetic loci in forest trees can be found in this volume (Neale and Tauer) and strategies proposed for another outbreeding organism (humans) in White et al. (1986) and Lalouel et al. (1986).

USE OF RFLP MARKERS IN FOREST GENETICS

For RFLP marker loci to be useful to tree breeders, they must be linked with phenotypes of economically important traits. Such linked markers can help to position the gene(s) controlling the expression of those traits on the genetic map. In this context it may be useful to consider the RFLP loci as X-variables and important economic traits as Y-variables. Assuming the availability of markers (X-variables), the basic problem for tree breeders reduces to one of generating the phenotypes (Y's) for traits of interest. By generating the required pedigrees and performing experiments that allow the clean expression of individual gene(s) as phenotypic variation among individuals within the pedigrees, breeders provide the critical information needed to utilize markers effectively to improve important traits.

Pedigrees

Pedigrees are important in understanding qualitative as well as quantitative trait variation. Quantitative trait variation is typically analyzed with methods based on the covariance among family members. The genetic model in these analyses specify that the trait under study is controlled by a large number of independent loci each with an equal and additive effect. It is becoming apparent that more complex models are needed to better fit the genetics of many quantitatively inherited traits (Kempthorne 1988). Pedigree analysis utilizes independent pedigrees to distinguish between monogenic and polygenic traits and in the case of monogenic traits allows estimates of parameters of the genetic model (Elston and Stewart 1971, Lange and Elston 1975). In the case of polygenic traits large computer simulations may be the only means of uncovering the action and interaction of the individual genes. For either case, multi-generation pedigrees with large family sizes will be most efficient for providing the necessary data.

Experiments

Having generated a set of multi-generation pedigrees it becomes necessary to test and score the individuals for one or more traits. RFLP markers seem to offer the most immediate application to the genetic understanding and improvement of simply inherited traits such as disease resistance. An example of such a trait in forest trees is major gene white pine blister rust

(Cronartium ribicola J.C. Fischer ex Rabenh.) resistance (MGR) in sugar pine (Pinus lambertiana Dougl.). A rare, dominant allele at the MGR locus provides blister rust resistance against a wide variety of rust races (Kinloch and Littlefield 1977). In the South, the most important disease of forest trees is fusiform rust (Cronartium quercuum (Beck.) Miyabe ex Shirai f. sp. fusiforme), and individual loci expressing resistance have not been identified. This is probably due to experimental methods, since most inoculation experiments have utilized, by design, heterogeneous rust (multiple gall cultures) and pine (open-pollinated families) sources.

However, Snow et al. (1975) may have found a rare resistant gene in a slash pine (Pinus elliottii Engelm.) tree of south Mississippi origin. Open-pollinated progeny of this tree were not infected (no gall formed) when separately inoculated with two single gall rust cultures also of south Mississippi origin. In the same experiment these single gall cultures were found to infect about 50% of the progeny in other "resistant" open-pollinated families. Griggs and Walkinshaw (1982) verified this result in a five-tree diallel cross involving the "resistant-gene" containing parent. All progeny of this tree, excluding 25% in one reciprocal, were free of infection when inoculated with one of the single gall cultures of Snow et al. Progeny of the other parents were infected to various degrees, 25 to 91 percent, when inoculated with this same inoculum. These cases are unusual, but they serve to illustrate the possibility of exposing single gene expression in progeny of forest trees, when spurious genetic variation in both host and pathogen is reduced.

An idealized experimental set-up for exposing single gene (host-pathogen) resistance responses can be envisioned for fusiform rust in southern pines. In this experiment, tested or presumed resistant and susceptible trees are mated to produce several pedigrees of n full-sibs. Each member of the sibship is then cloned to produce r ramets for each of m pathogen cultures. For each host by pathogen genotypic combination, the r ramets are inoculated with one of the m rust clones. The rust clones may be produced vegetatively or possibly by single urediospore cultures, or better yet, by inbreeding single urediospore cultures to homozygosity. The main features of this set-up are (1) control of spurious genetic variation in the rust by using homogeneous (and if possible homozygous) inoculum, (2) control of environmental variation in the inoculations by using k clonal pine replicates per inoculation, and (3) allowing multiple inoculations per pine genotype by using m*k clonal pine replicates per pine genotype.

Given the data from this experiment (Y's) and RFLP loci data (X's) pedigree analyses could provide insight on the genetic model and a map of the individual resistance loci. With RFLP and resistance loci mapped, simple marker assisted breeding procedures could be employed to increase the efficiency of tree breeding programs. The most likely areas of increased efficiency appear to be early selection and inter-species and inter-population gene introgression (Beckmann and Soller 1986, Tanksley et al. 1981). With a saturated map of RFLP loci, detailed investigations of quantitative trait inheritance become a reality (Lander and Botstein 1989, Paterson et al. 1988).

CONCLUSION

The new DNA technology has allowed geneticists for the first time to directly study variation in the DNA molecule itself, and to translate DNA polymorphisms between individuals into useful molecular marker loci. As these new molecular markers become readily available in forest tree species, as they almost certainly will, forest geneticists should be able to use these molecular markers to great advantage in their breeding and selection efforts. By developing advanced pedigrees and designing and installing the kinds of experiments required to expose gene action in these pedigrees, tree breeders provide critical information that will largely determine whether or not these new markers systems realize their potential for enhancing and accelerating the tree improvement process.

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INCORPORATION OF BIOTECHNOLOGY INTO TREE IMPROVEMENT PROGRAMS

J. P. van Buijtenen and W. J. Lowe[1]

Abstract.--This paper examines the techniques collectively referred to as biotechnology, the breeding strategies used by the Western Gulf Forest Tree Improvement Program (WGFTIP) and various ways of incorporating the new techniques into the overall program.

The main techniques of interests are new methods of vegetative propagation, gene transfer and expression, regeneration of viable plants from transformed tissue and the use of restriction fragment polymorphism (RFLP's). The main breeding strategies of interest are sublining of breeding populations and step-wise screening procedures. The incorporation of most techniques is rather straightforward and driven both by the availability of the necessary technology and economics. The issue of population improvement is more complex. Some of the issues that need to be resolved are discussed, although the best strategy is not obvious at this time.

Keywords: Breedign strategy, step-wise screening, sublines, vegetative propagation, molecular genetics, tissue culture.

INTRODUCTION

Recently a number of presentations have been given that discussed this topic, but most fail to address some of the important issues. An exception is the article by Riemenschneider et al. (1988), which gives a very thoughtful discussion of the topic. For the purpose of this discussion, the concept of biotechnology is not a very useful one. Biotechnology is not a discipline, but rather a fairly specific set of tools, which can be used for a variety of purposes. In the following discussion, we will not try to make a detailed description or exhaustive list of these techniques, but rather try to organize the available techniques into categories with application to tree improvement.

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The first broad category is the introduction and maintenance of genetic variation. Currently two very powerful techniques are available that contribute to this. One is the production of somaclonal variation. Somaclonal variation occurs in tissue culture, particularly callus culture, with the result that plants derived from the same ortet may show considerable variation. This variation can actually be transmitted sexually. The other technique is genetic transformation, also known as genetic engineering or gene splicing, in which a specific piece of DNA is inserted into a recipient cell. This technique allows the introduction of new genetic variations by inserting a desirable gene into a population. For instance, a gene for rust resistance could be inserted into a rust susceptible species such as slash pine Pinus elliottii Engelm. var. elliottii).

The next major category is selection. Here too biotechnology can contribute a couple of techniques. One example is in vitro screening procedures. Newton and van Buijtenen (1984), for instance, reported on procedures to screen loblolly pine genotypes for drought tolerance in vitro. The other technique involves the use of restriction fragment length polymorphisms (RFLP's). These are genetic markers, which can be used the same way as isozymes or morphological traits. They are different only because they can be produced in very large numbers, which makes it possible to develop very detailed chromosome maps. This, in theory makes it possible to do very early evaluations of genotypes. A number of things need to be known to accomplish this. First, one has to establish that a particular marker is closely linked to a locus controlling a desired trait. Second, one needs to determine whether the desired allele and the marker are in the coupling or repulsion phase. In the coupling phase the marker and the desirable allele are adjacent to each other, while in the repulsion phase the marker and the undesirable allele are adjacent. Once this is known, one can select progenies on the basis of presence (coupling) or absence (repulsion) of the marker.

Another major area of importance to tree improvers is genetic recombination. Again, biotechnology offers a technique here, although it is perhaps further from application than those mentioned previously. This is the technique of cell fusion. In theory, this technique enables the production of hybrid nuclei that could not be achieved by sexual means.

The final category is propagation techniques. Here, biotechnology offers plantlet regeneration via tissue culture (organogenesis) and plantlet regeneration via embryogenesis (Cheliak 1987). The success of these techniques is highly species dependent, but both techniques have been successful for some forest tree species.

Let us now look at the same situation for operational tree improvement techniques using the same broad categories.

OPERATIONAL TREE IMPROVEMENT TECHNIQUES

Introduction and Maintenance of Genetic Variation

The best opportunity to introduce genetic variation is at the beginning of the breeding program by establishing a broad genetic base. During the course of selection variation will be gradually diminished and the best one can do is what the politicians call damage control. By maintaining a large minimum population size and subdividing the breeding population, the probability of losing genetic variation is reduced, but not eliminated. One can also introduce new material into the breeding population, but after several generations of selection this will partially offset the progress already made.

Selection

Here we have a number of well established techniques, such as first generation selection, progeny testing and second generation selection. New techniques being developed now include early testing and step-wise screening. In early testing a trait is selected at the seedling stage which is reasonably well correlated with future performance. Total above ground dry weight is looking very promising (Lowe and van Buijtenen 1989). Other traits, such as drought resistance or rust resistance can be naturally tested at an early age. Step-wise screening is another procedure that we have used successfully. It consists of evaluating a number of families in a series of tests, each eliminating part of the families. By using the more economical tests first, a great reduction of progeny testing costs can be achieved. We have, for instance, selected longleaf (Pinus palustris Mill.) in short-term tests (three years) for their ability to survive and emerge from the grass stage quickly. The best families from the short-term tests are then established in a long-term test to observe their growth and form.

Genetic Recombination

This is, of course, accomplished by inter-crossing. It is reasonably safe to consider the partial diallel as the ultimate mating design, primarily because it is virtually impossible to successfully complete a full diallel and anything less is a partial diallel.

To control inbreeding it has proved advantageous to sub-divide the population into breeding groups or sublines (Lowe and van Buijtenen 1986). All crossing for breeding purposes is carried out within the breeding groups, while in the seed orchards, the design is such that crossing is almost exclusively among breeding groups. This implies the other major consideration which is the separation of the breeding population and the production population. The breeding population is contained in the scion banks and progeny tests and the production population is the seed orchard. Generally speaking, only the very best trees out of the breeding population are included in the seed orchards.

Propagation Techniques

Many good techniques are available, the success of which is highly species dependent. Sexual propagation in scion banks and seed orchards is usually the

most economical way to mass produce desired genotypes. Grafting techniques have been developed for many species, but the cost is usually too high for mass production. Rooted cuttings can be produced readily for some species such as poplars, spruce, and eucalyptus, but with much greater difficulty (or not at all) for other species such as pines and oaks.

INTEGRATION OF THE TWO SETS OF TOOLS

We just developed two separate lists of tools serving the same four major purposes. So now, how do we integrate the two? The answer, of course, is very simple, combine the two lists, as shown in Table 1. On the surface, this seems

Table 1. Combined list of currently operational tree improvement techniques and biotechnologies useful for breeding.

Introduction and Maintenance of Genetic Variation
Use of exotics
Selection in variable species
Crosses among diverse parents
Somaclonal variation
Genetic transformation
Selection
First generation selection
Progeny testing
Advanced generation selection
Early testing
Drought resistance
Rust resistance
Shoot dry weight
In vitro screening procedures
RFLP's
Genetic Recombination
Partial diallel mating
Cell fusion
Breeding group (sublines)
Separation of breeding and production populations
Propagation Techniques
Rooted cuttings
Tissue culture plantlets
Organogenesis
Embryogenesis
Grafting
Seed orchards

too easy and some nasty minds might accuse me of having been an administrator too long and learned how to play these slick paper tricks. So, let us consider this in more depth. Figure 1 shows the breeding cycle as we currently practice it. Initially a base population was established of first generation selections. New materials can still be introduced from outside the cycle, although this becomes increasingly difficult as the selected material increases in quality. The initial or introduced material then goes through the selection and testing phase, including step-wise screening. From there, the selected material is mated and the new materials are propagated for another round of selection and testing. The breeding population may contain anywhere from 300 to 3000 genotypes. Out of these the best ones will be selected to go into the seed orchards, typically, 10 to 50 genotypes. The seed orchard produces the improved seed which goes to the nursery and is then planted on a large scale. As you all know, this amounts to about a million acres annually in the south. Under some circumstances it is possible to take the best few genotypes and mass produce them using rooted cuttings.

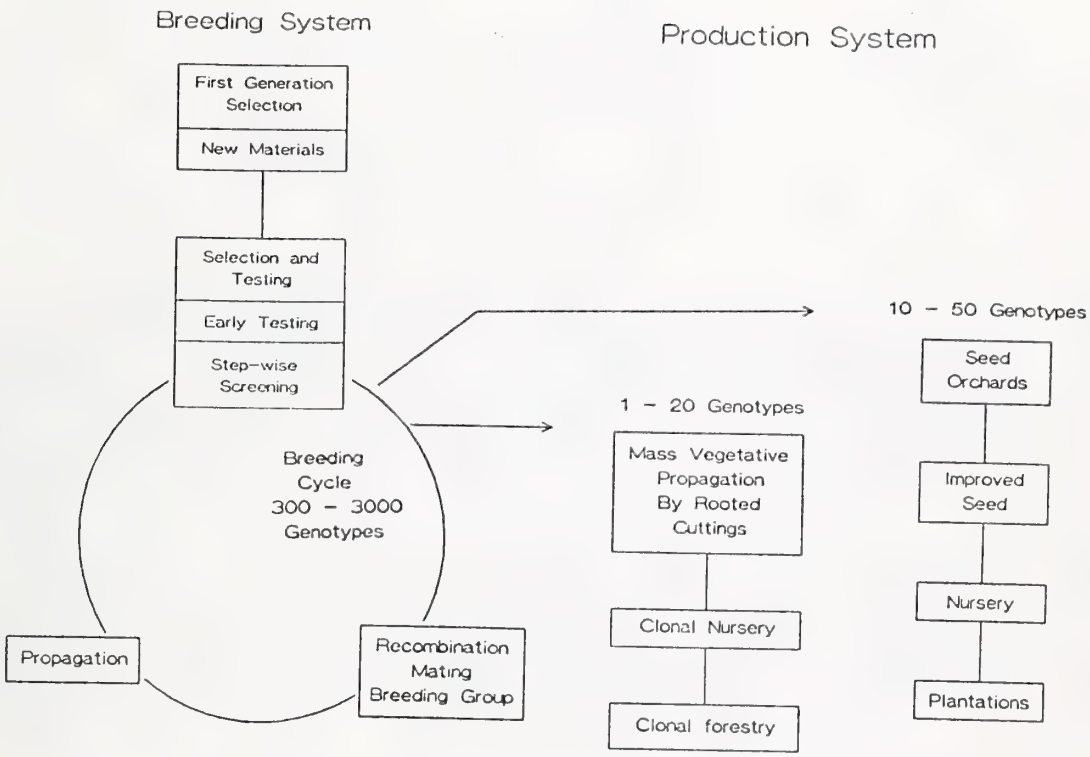

Figure 1. Tree Improvement Using Current Technology

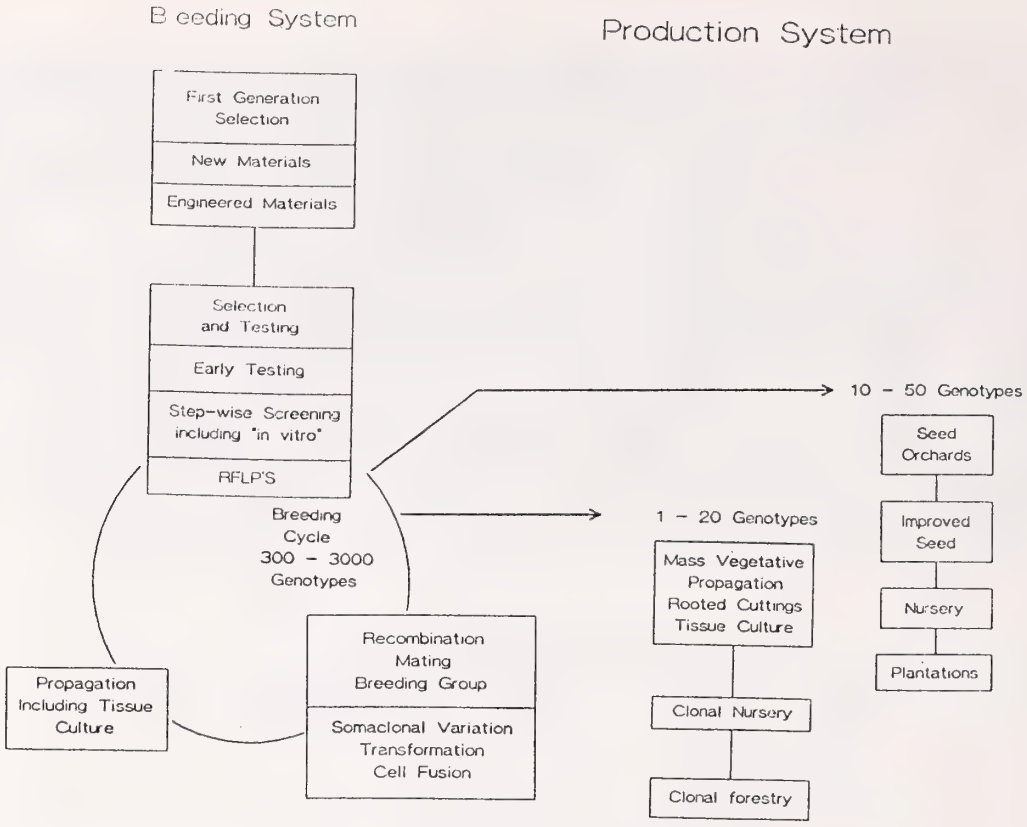

Figure 2. Tree Improvement Including Biotechnologies

Now, let us look what is changed when biotechnologies are added (Figure 2). First of all, at the introductory step, other materials, such as engineered materials can be added and fed into the system. Materials can also be introduced during the recombination phase. At the introductory phase, the materials are essentially unrelated to the existing population. They should go through the selection and testing steps. This might include in vitro early tests and RFLP's to assist early screening. In the recombination phase, somaclonal variation, transformation, and cell fusion might be used to introduce new variation. This is a powerful new addition to the breeding cycle. Previously we could only minimize the loss of genetic variation. Now we have added some promising tools to maintain or actually increase genetic variation. This material then goes through a propagation phase, which might include tissue culture techniques. Next it must be evaluated in the selection and screening phase, after which the cycle repeats itself. The number of genotypes used should stay about the same as before. The seed orchard phase is essentially unchanged, and material can be deployed in seed orchards as it was before.

The capabilities of vegetative propagation have also been expanded, since it now is possible to produce plantlets via tissue culture using techniques based on organogenesis or embryogenesis. These can be used alone or in combination with rooted cuttings, such as the system used in New Zealand with radiata pine.

A HYPOTHETICAL EXAMPLE

For illustration, let us use an example that is not too far fetched. Assume that someone introduces a gene for Roundup®[2] resistance in Virginia pine. This would be of considerable benefit to the Christmas tree growers since weed control is extremely important in Christmas tree production. Christmas tree growers can also pay more for a seedling than a forester and the somewhat expensive techniques might be economical in Christmas trees before they would be economical in operational forestry. Let's further assume that the gene has been introduced in one of the best individuals available, in other words in a tree that is already part of the breeding cycle. What steps would follow?

1) First, the materials should be put in a short-term test to verify that it indeed possesses Roundup® resistance.
2) It needs to be put into a long-term test to verify field performance.
3) It could be mass propagated vegetatively for sale to Christmas tree growers,
4) It could be introduced into the breeding population by crossing it to other outstanding parents, and
5) The clone could be included in a seed orchard.

There remain number of questions to be answered. One is the question of economics. Currently, Christmas tree seedlings sell for $50 to $60 per thousand. However, a grower could afford perhaps up to $1 per seedling ($1000 per thousand) if a tree was really exceptional, which means it had good quality and shortened the rotation age by one year. This technology would seem fairly realistic. For instance, one organization currently is selling rooted loblolly pine cuttings at a cost of $150 per thousand.

For forestry operations the economics look somewhat different. The current cost of seedlings is about $30 per thousand. One percent gain in volume growth is worth about $4 per acre. Assuming that gain by genetic engineering is as high as 50 percent and 500 trees are planted per acre, a seedling cost of about $400 per thousand could be justified. Of course this would not result in any profit for the grower so the cost should probably be no more than half of that, perhaps $200 a thousand. This is again in the feasible range, although the gain required to justify this cost may to difficult to achieve.

Another major question centers around the introduction of engineered genes into the breeding population. I'm not aware that anyone has addressed this question so far. Personally, I would like to introduce more than one copy of a desirable gene into the population, so how would one go about it? Would one introduce this gene in all 24 trees per breeding group? This might not be as difficult as it seems. If it can be done in one tree, it might not be that much

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[2] Mention of trade names is solely to identify material (or equipment) used and does not imply endorsement by the Texas Forest Service. If pesticides are mentioned, this does not imply recommendations for their use, nor does it imply that the discussed uses have been registered. All uses of pesticides must be registered by appropriate State or Federal agencies before they can be recommended.

more complicated to do it in 24. The most difficult part is usually getting the procedure to work the first time. If this is successful, however, current technology will insert the gene at 24 different loci. This may not necessarily be bad. After repeated mating, many trees will wind up with multiple copies of the introduced gene. Since most likely, there will be a dosage effect, this means that the resistance to Roundup might continue to increase after several cycles of selection. It also could mean that any unfavorable side effects would continue to accumulate. In the long run, selection would probably favor an optimal dosage.

From the foregoing it appears that, at least conceptually, there are no major problems integrating biotechnologies with the technologies that are currently operational. From a practical point of view it might, however, not be easy. Developing the technologies to the point where they can be applied routinely and economically will be a major challenge. There are also some organizational problems. Currently, biotechnologists and tree improvement specialists work at best on two different teams. On occasion I have received the impression they are working on competing teams. Neither situation is conducive to integration of the two technologies. As scientists we can alleviate the situation somewhat by being aware of what is happening in both worlds. The problem is, however, primarily an organizational one, and I expect it will soon be profitable to create tree improvement teams with biotechnologists, physiologists, geneticists and breeders as members.

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ESTABLISHMENT OF THE SECOND-GENERATION SELECTED POPULATION OF SLASH PINE IN THE COOPERATIVE FOREST GENETICS RESEARCH PROGRAM

G.R. Hodge, G.L. Powell, and T.L. White[1]

Abstract.--A total of 886 individuals have been selected to comprise the second generation of improved slash pine in the Cooperative Forest Genetics Research Program (CFGRP). The bulk of the second-generation selections were made from a base population which included a total of 2373 original first-generation selections and the best offspring from each of 2700 full-sib crosses among first-generation parents. Best linear prediction was used to predict breeding values for volume and fusiform rust resistance, and the 5073 candidates were ranked according to their predicted breeding values. The primary objectives in the second-generation selection were: 1) to maximize genetic gain for the economically important traits of volume growth and rust resistance, and 2) to maintain a broad genetic base. Of the 886 second-generation selections, 304 were selections of the best offspring from the best full-sib families (forward selections). Within family selection in the field was aided by screening progeny test data by computer. A total of 404 selections were the best original first-generation parents (backward selections) which were retained for the second generation. In addition a total of 178 selections which generally had not been tested in the field were infused into the second-generation population. These infusions were generally superior for traits of special interest such as a high degree of rust or pitch canker resistance, or gum production. The 886 selections have been assigned to 24 breeding groups with approximately 35-40 selections in each group. All relatedness is confined within breeding groups. Each member of the CFGRP is responsible for clone banking, breeding and testing one or two breeding groups. To date 90% of the 886 second-generation selections have been grafted into clone banks where breeding will be conducted.

Keywords: *Pinus elliottii*, breeding strategy, forward selection, backward selection.

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INTRODUCTION

The Cooperative Forest Genetics Research Program (CFGRP) at the University of Florida began mass selection of slash pine (*Pinus elliottii* Engelm. var *elliottii*) from unimproved natural stands in the mid-1950's. These selections were grafted into first-generation seed orchards, and progeny tests of these selections were established using wind-pollinated orchard seed. Numerous full-sib crosses between first-generation parents were made and full-sib progeny tests established for the purpose of selecting the best individuals within the best full-sib families. Over the past three years, the CFGRP has created the second-generation selected population for slash pine and is nearly finished grafting these selections into clone banks. This paper describes the process which proceeded as follows: 1) formation of the second-generation population on paper, 2) delineating breeding groups, 3) making forward selections in the field, and 4) establishment of clone banks.

FORMATION OF THE SECOND-GENERATION SELECTED POPULATION

Objectives

The main objectives guiding the formation of the selected population were to 1) maximize genetic gain, and 2) maintain a broad genetic base for continued improvement in future generations. This meant selecting the best clones in the program for inclusion in the population with some constraints on relatedness of the selections. Material potentially available for inclusion in this second-generation selected population consisted of: 1) 2373 original first-generation selections, called backward selections, 2) forward selections of the best individuals made from 2700 different crosses in full-sib tests, and 3) a number of promising (but basically untested) clones which could be included as infusions into the population.

Ranking of Candidates

The first step in forming the population was to rank all possible candidates in the CFGRP according to their predicted genetic value. Based on progeny test data, breeding values for volume and rust resistance of original first-generation parents were predicted using best linear prediction (BLP) (White and Hodge 1988). We also wished to make forward selections of the best individuals from the best of some 2700 different full-sib families which have been established in cross tests over the last 15 years. Breeding values for forward selections were estimated as the mean of the parental breeding values plus an incremental increase in breeding value from within family selection (BV_W). Only one selection was made from a full-sib family, and BV_W was calculated assuming selection of the tallest rust-free individual in a single progeny test with 30 trees per full-sib cross. Breeding values of forward selections calculated in this manner were directly comparable to breeding values of the first-generation parents, thus first-generation parents and forward selections could be ranked together.

Using a growth and yield program that quantifies the effect of rust infection on yield (Nance et al. 1983), economic weights for volume breeding

value (BVVOL) and rust breeding value (BVR50) were estimated. Since the economic weight estimates are rather imprecise, and the importance of the two traits may differ among cooperative members (and over time), three indices were developed that have different relative weights on growth and rust:

$$I_B = 0.5\ BVVOL + 0.5\ (50 - BVR50)/100,$$
$$I_G = 0.9\ BVVOL + 0.1\ (50 - BVR50)/100, \text{ and}$$
$$I_R = 0.1\ BVVOL + 0.9\ (50 - BVR50)/100$$

where I_B = aggregate genotypic value when rust and growth are given their estimated economic weights,
I_G = aggregate genotypic value with primary emphasis on growth, and
I_R = aggregate genotypic value with primary emphasis on growth.

All 5073 candidates for the second-generation population (2373 original first-generation selections and a forward selection from each of 2700 full-sib crosses) were ranked according to these three indices.

Forward versus Backward Selections

The 2700 full-sib crosses made by the CFGRP were formed more or less at random (from a genetic quality standpoint). Thus the best of the 5073 candidates (ranked by any of the three indices) which were available included a mixture of forward and backward selections. In forming the second-generation selected population, there was no inherent advantage in choosing forward selections versus backward selections. In some cases, it may be better (from the standpoint of making genetic gain) to include an outstanding backward selection, say parent A, in the population as opposed to an average, or even above average offspring of A crossed with another parent, say B, that is inferior to A. The objectives were to maximize genetic gain, while maintaining sufficient genetic diversity. If parent A is the best single genotype currently available, it makes sense to carry that genotype on into advanced generation breeding.

In the absence of positive assortative mating, the best original first-generation parents in a breeding program are invariably mated only with other parents which are substantially inferior. This is simply a result of sampling: outstanding parents are rare, so if parents are mated at random it is unlikely that two outstanding parents will be mated. This generally results in the scenario described above: the expected breeding value of the best forward selection from a full-sib family of an outstanding parent crossed with an average parent is lower than the breeding value of the outstanding parent. This underscores the importance of assortative mating for future generations (Lindgren 1986).

Constraints on Relatedness

Generally, if a candidate was outstanding for any of the three indices, it was included in the selected population. However, during the selection process an attempt was made to balance the objectives of maximizing genetic gain and minimizing relatedness in order to maintain a broad genetic base. Thus, the maximum number of relatives allowed was 5, and this number decreased with decreasing predicted genetic value. For example, a parent (say parent A) with

very high aggregate genotypic values (I_B, I_G, I_R) was allowed to contribute genes to the selected population by inclusion of the parent itself (the original selection A) and inclusion of one forward selection from each of two to four full-sib crosses of A with other parents (i.e., up to 5 relatives). In contrast, an above average but not outstanding parent (say B), might be included in forward selections from two full-sib crosses of B with other parents, or a selection from one full-sib cross and the original selection (i.e., two relatives). Finally, an average parent (C), was usually included as only one forward selection from a full-sib cross of C with a better parent.

Final Composition of the Selected Population

The second-generation selected population for slash pine currently has 886 members (Table 1). Note that 404 members of the population are original first-generation selections (i.e., backward selections) whose predicted aggregate breeding values (I_B, I_G or I_R) were high enough to warrant inclusion. Similarly, 304 members of the population are forward selections from the better of the 2700 full-sib families. The 178 infusions come from a variety of sources and, for the most part, were not ranked according to I_B, I_G and I_R. Their purpose is to broaden the genetic base of the selected population. For example, pitch-canker-free selections from highly infected stands were included in this selected population. The only trees listed as "infusions" were those that were not included on the basis of I_B, I_G or I_R. Overall, a total of 826 unrelated individuals contributed genes to the second-generation selected population, thus giving this population a broad genetic base (objective 2). However, substantial genetic improvement (objective 1) was also made as a result of the second-generation selection. After first-generation mass selection, there was a 10% gain in volume and no gain in rust resistance over unimproved checks (Hodge et al. 1989). Average predicted breeding values for the second-generation selected population indicated a 20% gain in volume over unimproved (volume per tree at 15 years in row-plot tests), and substantially increased rust resistance (e.g. 34% rust infection expected when unimproved material would incur 50% infection). Use of the best available clones in a production seed orchard would result in significantly higher genetic gain in both traits.

Table 1.--Sources of selections in the CFGRP second-generation slash pine selected population.

Backward selections (first-generation selections)		404
Forward selections from cross tests		304
Infusions		178
Rust-free selections	127	
High-gum selections	33	
Pitch-canker-free selections	8	
Rhodesian slash pine program	10	
TOTAL		886

DELINEATING BREEDING GROUPS

Genotypes (clones) in the selected population were divided into 24 different breeding groups of approximately 37 clones each. Breeding groups allow the management of inbreeding while still allowing establishment of outcrossing production seed orchards of unrelated genotypes (van Buijtenen and Lowe 1979, McKeand and Beineke 1980). Each cooperator is responsible for the breeding and testing of all genotypes in either 1 or 2 different breeding groups. Genotypes within a breeding group will be bred and tested together as a unit. There will be no breeding among genotypes in different breeding groups. All related selections (e.g., two selections with a common parent) were always assigned to the same group. For the most part, the 24 groups have similar average breeding values for rust resistance and volume growth. The infusions (e.g., pitch canker free selections, high gum selections and rust free selections) were distributed across groups.

FIELD SELECTION OF FORWARD SELECTIONS

Computerized Selection of Individuals

Much preparatory work was done by computer in order to maximize efficiency of selection and minimize the time spent in the field. Once it had been determined that forward selections from particular full-sib families would be included in the selected population, the CFGRP data management system was used to generate a second-generation selection form for each forward selection. First, all tests that the particular full-sib family occurred in were identified. The test in which to make the forward selection was then chosen to 1) maximize the effectiveness of selection, and 2) minimize the number of different tests to be visited during the selection process. Selection for growth was most efficient in the oldest tests, while efficiency of selection for rust resistance was maximized in tests with high infection. Using the most recent field measurements of a particular test, the five tallest rust-free trees (all heights standardized by block) were chosen as possible candidates for each full-sib family. These candidates were identified on the selection form by their block and position location in the progeny test.

There was some concern that the computer selection approach might tend to identify trees with missing neighbors thus decreasing the effectiveness of selection. Height growth is generally considered relatively independent of spacing (hence it is used as a measure of site index), but a small study was conducted to examine the effects of missing neighbors on height growth in 5 to 12-year-old progeny tests of slash pine. No significant effect was found, and therefore there is no reason to suspect that trees with missing neighbors were preferentially selected as candidates (White et al. 1988).

Final Field Screening

The second-generation selection forms were distributed to the CFGRP member who maintained the progeny test where a particular selection would be made. Candidate trees were ordered by standardized height and examined sequentially for 'acceptability' for three characteristics: 1) freedom from fusiform rust

infection (selection was often made one to three years after last measurement, and there was a possibility of subsequent rust infection), 2) straightness, 3) crown, primarily forking or ramicorn branching. Using a low selection intensity (i.e., acceptability) for straightness and crown form allowed for a much higher selection intensity for the more important trait of height growth.

Field selection using this technique was quite efficient. It was rarely necessary to examine more than the first three candidate trees to find a selection.

ESTABLISHMENT IN CLONE BANKS

In early 1988 the CFGRP began grafting the second-generation slash pine selected population into clone banks. These clone banks will serve to: 1) provide scions to establish production seed orchards with elite clones, 2) provide a location to make crosses for second-generation breeding and testing, and 3) preserve all genotypes of the population.

Once breeding groups were established on paper, it was clear that a great deal of scion exchange among cooperators would be necessary to get a selection from one cooperator's test or orchard to the cooperator responsible for its clone banking, breeding and testing. In order to facilitate future breeding and progeny testing of this selected population, an effort was made to complete the clone establishment in a two-year period (1988 and 1989). The following procedures were used in both years.

Prior to scion collection and grafting, the CFGRP data management system was used to generate lists tailored to each cooperator's needs. First, each cooperator was provided with the list of forward, backward and infusion selections from which to collect scions to be distributed to other cooperators. Also, each cooperator was provided with a list of all clones from which they were to receive scions collected by other cooperators. Standardized scion collection procedures were used, and all cooperators collected scions the last week in January in both years. On an assigned day, each cooperator then brought their scions to Tallahassee, FL, and exchanged them with other cooperators for scions of clones to be grafted into their clone banks. These scions were grafted during the first week of February.

At completion of grafting in 1989, CFGRP cooperators had made 15,052 grafts of 834 second-generation selections. The original target called for six ramets of each selected clone to be planted in a clone bank; a selection is considered "established" if at least four ramets survive after one year in the field. By the end of the summer of 1989, approximately 90% of the second-generation selections will be successfully established in clone banks. By the end of 1990, we anticipate that all 886 second-generation selections will be established in clone banks. At completion, each clone in the selected population will be preserved in two different locations: a primary location (clone bank or seed orchard) owned by the cooperator responsible for breeding that genotype, and a secondary location maintained by a different cooperator to ensure against loss.

SUMMARY

The 886 member second-generation selected population will serve as the basis for continued improvement of slash pine (through breeding and testing) and will also provide material for 2.0 and 2.5-generation seed orchards. The population includes backward selections (original first-generation parents), forward selections (offspring from full-sib crosses of first-generation parents), and infusion selections (promising but untested clones, or clones with special traits). The population has a broad genetic base, and substantial genetic gain resulted from selection. For example, average offspring from the second-generation selected population would incur 34% rust in areas where unimproved material would incur 50% infection, and yield approximately a 20% gain in volume over the unimproved check. Substantially more gain is possible by taking the best members of the population for use in a production seed orchard.

The second-generation formation and clone bank establishment was a major undertaking by the CFGRP. In two years, the cooperative identified 886 second-generation selections and then collected scion, grafted, and established 834 of these in clone banks. By the end of 1989, approximately 90% of these second-generation selections will be established in clone banks. In the winter of 1990, the cooperative will graft the final 52 selections and re-graft those selections with poor clone bank survival.

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GENERAL SESSION:
SEED ORCHARD MANAGEMENT

MATING PATTERNS IN SEED ORCHARDS

W. T. Adams and D. S. Birkes[1,2/]

Abstract.--Pollen contributing to the production of viable orchard seed comes from three sources: self-fertilization, gene flow from surrounding stands (contamination), and cross-fertilization with mates within the orchard. Understanding the relative contributions of each of these sources is critical to the development of orchard designs and management regimes that will maximize the genetic efficiency of orchard crops. In this paper, methods of evaluating mating patterns from the segregation of isozyme genetic markers in the seed of individual mother trees are briefly described and empirical results for conifer orchards reviewed. Self-fertilization accounts, on average, for less than 10% of orchard offspring, but the proportion of self progeny can vary substantially among individual clones. Pollen contamination can be responsible for as much as 50% of orchard seed and is probably the most serious impediment to the genetic quality of orchard crops. The limited data currently available on patterns of cross-fertilization within orchards suggest that near neighbors account for only a small percentage of the offspring produced by an individual ramet, and with low pollen contamination, cross-fertilization among clones may be extensive.

Keywords: Mating patterns, seed orchard, isozyme, pollen contamination, selfing, cross-fertilization.

INTRODUCTION

Patterns of mating in seed orchards determine the parentage of crosses and thus, the genetic quality and variability of seed that is produced. Pollen successful in fertilizing the offspring of any individual mother tree or ramet can be attributed to three sources: self-fertilization, migrant pollen from outside the orchard (gene flow or pollen contamination), or cross-fertilization with other males in the orchard.

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2/ Paper 2565 of the Forest Research Laboratory, Oregon State University.

The proportion of seed due to selfing is significant because of the usual poor survival and growth of selfed offspring (Franklin 1970, Sorensen and Miles 1982). Although the proportion of selfed offspring generally appears to be low (<10%) in natural stands (Adams and Birkes 1989), it might be expected to be higher in seed orchards due to the wide spacing of individuals; and in clonal orchards, due to "effective selfing" resulting from crosses among ramets of the same clone.

Levels of pollen contamination are of concern because seed resulting from fertilization by non-orchard pollen is expected to have only one-half the genetic gain of seed fertilized by orchard parents. Reduction in gain may even be worse if contaminant pollen comes from trees poorly adapted to the intended planting sites of orchard seed.

Patterns of cross-fertilization among orchard parents are important in determining the amount of genetic variability produced in seed crops. In addition, the relationship between mating success and distance between mates must be known so that minimum distances needed to prevent mating between related individuals can be incorporated into orchard designs (Marsh 1985). Rapid reduction in the amount of pollen dispersed from individual trees with increasing distance (Levin and Kerster 1974), suggests that most mating in seed orchards may be between near neighbors. Large differences among clones in pollen fecundity and in the timing of pollen shedding, however, may override distance as factors influencing mating success (Shen et al. 1981, Erickson and Adams 1989).

The ability to study mating patterns in plants has been enhanced in recent years by the availability of isozyme genetic markers (Brown et al. 1985, Adams and Birkes 1989). Segregation of these markers in the offspring of mother trees with known genotype reflects actual patterns of mating in the previous generation. Mating models developed to account for the genotypic segregation in progeny can be applied to observed offspring arrays and the parameters from the models estimated. Conifers are particularly amenable to mating model studies, because by assaying both the haploid megagametophyte and diploid embryo of a seed, the isozyme genotype of the pollen gamete which fertilized that seed can be determined (Adams 1983).

In this paper, we review results of mating parameter estimation in conifer seed orchards that are wind-pollinated and have been subjected to a minimum of pollen management, other than the possible presence of a pollen dilution zone surrounding the orchard. In practice, the three components of mating mentioned above have been studied using simplified models that address each component separately. Statistical procedures for estimating levels of selfing and pollen contamination are adequately described in the literature, so for these two components of mating, we will emphasize the results of parameter estimation. Little data is yet available on cross-fertilization patterns in seed orchards. For this aspect of the breeding system, more effort will be devoted to describing models and estimation procedures.

SELFING VERSUS OUTCROSSING

The greatest emphasis in mating pattern studies has been in determining the proportion of viable progeny produced by selfing versus outcrossing. A variety of estimation procedures, utilizing either single-locus or multilocus progeny arrays have been employed, but they are all based on the same simple model, the mixed-mating model (Brown et al. 1985). This model assumes that all offspring are the result of either selfing (with probability s) or random outcrossing to other trees in the population (t=1-s). Results are usually reported in terms of estimated rates of outcrossing, $\hat{t}$.

Current orchard designs emphasizing large numbers of clones (i.e., >25), and physical separation between ramets of the same clone, appear to be effective in generally limiting the frequency of viable selfs. On average, proportions of outcrossed progeny in clonal seed orchards have been found to be high (Table 1), usually greater than 0.90, and no lower than observed in natural stands of the same species.

Table 1. Estimated proportions of progeny in clonal seed orchards resulting from outcrossing ($\hat{t}$)

Species	Location	Loci[a]	$\hat{t}$[b]	SE($\hat{t}$)	Reference
black spruce	Ontario	5	.837	.037	Barrett et al. 1987
Douglas-fir	Oregon	11	.910	.020	Shaw and Allard 1982
	Oregon	10	.936	.026	Omi and Adams 1986
loblolly pine	South Carolina	7	.987[c]	.008	Friedman and Adams 1985a
Monterey pine	Australia	4	.900[d]	.040	Moran et al. 1980
Scots pine	Sweden	7	.957	.004	El-Kassaby et al. 1989
	Sweden	7	.961	.003	" "
	Finland	7	.976	.017	Muona and Harju 1989
	Finland	7	.978	.017	" "

[a]Number of polymorphic loci used in estimating t.
[b]All estimates except for Monterey pine are based on multilocus estimation procedures. The estimate for Monterey pine is an average of single-locus estimates of t.
[c]Unweighted mean over two orchard blocks and two crop years.
[d]Unweighted mean over three crop years.

Considerable variation in t, however, can occur among individual ramets or clones. In Douglas-fir orchards, individual outcrossing rates as low as 0.50 have been observed (Shaw and Allard 1982, Erickson 1987). Two main

factors are responsible for variation among individual trees in t. The first is the relative availability of self versus outcross pollen at the time of fertilization. Extremely early or late flowering clones, or particularly heavy pollen producers, might be expected to have higher proportions of self-pollen when female strobili are receptive, and thus, have lower frequencies of outcrossed progeny (El-Kassaby et al. 1988, Erickson 1987). Clones also differ in the ability to produce viable embryos after selfing (i.e., self-fertility), mostly as a function of the number of lethal-gene equivalents they possess (Sorensen 1969). Normally, self-fertility is quite low in conifers (Franklin 1970), but it can range extensively among individuals. For example, in a study of 35 Douglas-fir trees, Sorensen (1971) found self-fertility to range from 0.1 to 45 percent. With equivalent frequencies of self-fertilization, proportions of viable self progeny will be highest in clones with the highest self-fertilities.

POLLEN CONTAMINATION

The use of single-gene markers to identify seeds fertilized by non-orchard pollen sources is limited because allele frequency differences between orchards and background stands are usually small. A multilocus method of estimating gene flow, however, can be applied in clonal orchards (Smith and Adams 1983, Friedman and Adams 1985b). This procedure takes advantage of the fact that clonal orchards have a limited number of multilocus genotypes relative to background stands. Thus, background pollen sources are expected to produce a much greater variety of multilocus gametes which collectively can be used to identify migrant pollen. Pollen gametes observed in orchard offspring which have multilocus genotypes different from any that can be produced by the orchard clones are called detectable contaminants. The proportion of detectable contaminants (b) in an offspring sample provides a minimum estimate of pollen contamination, since not all migrants will be detectable genetically. To obtain an estimate closer to the true value, b must be divided by the probability that a migrant pollen grain will have a detectable genotype (i.e., the detection probability). The detection probability can be estimated from allele frequencies of trees sampled in background stands (Smith and Adams 1983).

Estimates of pollen contamination ($\hat{m}$) based on the multilocus procedure have been made for a variety of species and orchard situations. The estimates in Table 2 are for total contamination, including migrant pollen from natural stands, and from other orchard blocks on the same site. In all cases, the levels of pollen contamination appear to be quite high. In one young Douglas-fir orchard with poor pollen production, $\hat{m}$ was greater than 90 percent. But even in older orchards, where pollen production is much greater, contamination has been observed to be as high as 40 to 50%. Fortunately, pollen management techniques such as flower stimulation, supplemental mass pollination, and bloom delay (by water cooling) appear to be effective in reducing contamination in seed orchards (El-Kassaby and Ritland 1986, Wheeler and Jech 1986).

Table 2. Estimates of pollen contamination ($\hat{m}$) in conifer seed orchards based on multilocus genetic markers.

Species	Orchard Location	Block size(ha)	Age	$\hat{m}$	SE($\hat{m}$)	Reference
Douglas-fir	Oregon	1.8	14	0.52[a]	0.02	Smith and Adams 1983
	Oregon	2.4	20	0.40[b]	0.05	"
	Oregon	3.3	8-9	0.91[b]	0.08	Adams, unpublished
	Washington	5.0	15	0.44[c]	0.04	Wheeler and Jech 1986
loblolly pine	South Carolina	2.0	15	0.46[d]	0.02	Friedman and Adams 1985b
	Texas	-	-	0.51[b]	0.05	Wiselogel 1986
Scots pine	Finland	-	-	0.36[b]	-	Harju et al. 1987
	Sweden	12.5	17-18	>0.36[b,e]	-	El-Kassaby et al. 1989
	Sweden	16	14-18	>0.21[b,c]	-	"

[a]Mean estimate for 10 orchard blocks in one cone crop year.
[b]Estimate for one orchard block in one cone crop year.
[c]Mean estimate for two orchard blocks in one cone crop year and one orchard block in a second crop year.
[d]Mean estimate for two orchard blocks in three crop years.
[e]A minimum estimate, based only on the proportion of detectable contaminants (see text).

PATTERNS OF CROSS-FERTILIZATION WITHIN ORCHARDS

Two approaches have been employed to investigate cross-fertilization mating patterns in seed orchards using genetic markers. In the rare marker approach, offspring are sampled from mother trees at varying distances and directions around a pollen parent carrying a rare marker allele (Shen et al. 1981, Erickson and Adams 1989). The frequency of the rare marker in the offspring of each mother tree is a measure of the relative mating success of the pollen parent. This is an effective means of measuring cross-fertilization patterns, particularly the relationship between mating success and distance between mates, but is limited in application because of the requirement for individuals with rare markers. In addition, the presence of multiple ramets carrying the same rare marker, can confound the interpretation of results.

In the model approach, mating parameters are estimated directly from probability models designed to account for the frequencies of multilocus genotypes of pollen gametes in the offspring of individual mother trees. This approach was used by Schoen and Stewart (1986) to investigate male

fertilities of clones in a white spruce orchard. A more general model has also been described by Roeder et al. (1989). Both of these models, however, require that populations be isolated from migrant pollen sources.

As is evident from the previous section, it cannot be assumed that orchards are isolated. Thus, there is a need for methods of evaluating cross-fertilization patterns when at least some pollen contamination can be expected. A probability model which can be used under such circumstances is the "Neighborhood Model" described by Adams and Birkes (1989). In this model, a specified area around a mother tree is called its neighborhood. The model states that pollen successful in fertilizing viable offspring of the mother tree is due to three sources: self-fertilization (with probability s); migrant pollen from outside the neighborhood (with probability m); and, cross-fertilization with males within the neighborhood (with probability 1-m-s), with the relative mating success of the jth outcross male in the neighborhood being ϕ_j. The probability, therefore, of observing multilocus genotype g_i in the pollen gametes of offspring from the mother tree is

$$P(g_i) = s\ P(g_i|M) + (1-m-s) \sum_{j}^{r} \phi_j\ P(g_i|F_j) + m\ P(g_i|B),$$

where $P(g_i|M)$ is the probability that the mother tree produces pollen gametes with genotype g_i, $P(g_i|F_j)$ is the probability that the jth outcross male in the neighborhood produces pollen gametes of this type, and $P(g_i|B)$ is the probability that pollen gametes from migrant (background) sources have genotype g_i.

The relative mating success of each outcross male in the neighborhood (ϕ_j) is determined by a number of factors including proximity to the mother tree, pollen fecundity relative to other males, and the degree to which the pollen shedding period overlaps with the female receptivity period of the mother tree. To investigate the relationship between mating success and factors expected to influence mating success, ϕ_j can be expressed as a function of these factors. Thus, the parameters to be estimated in the model are s, m, and one or more terms related to mating success within the neighborhood. Maximum likelihood procedures can be used to estimate these parameters when the model is applied to offspring data. In practice, the neighborhood might be an entire orchard block, but in clonal orchards it is necessary to keep neighborhood size relatively small, because as neighborhood size increases, the number of individuals with identical genotypes within the neighborhood increases. The smaller the number of potential males within the neighborhood, the greater the ability to discriminate among them genetically, and between multilocus pollen gametes from within and outside the neighborhood.

We have applied the neighborhood model to offspring data from five ramets in a 20-year-old block of a 25-clone Douglas-fir seed orchard (Adams and Birkes 1989). All pollen producing trees within a 25-m radius (25-26 trees) were considered the neighborhood of each mother tree ramet. Genotypes of all trees in the block were known at 11 loci, and estimates of pollen gamete frequencies in background pollen sources were also

available for the same loci. In addition, pollen gamete genotypes were available for samples of 30-40 offspring per mother tree.

First, we were interested in the relationship between mating success (ϕ) of the males in a neighborhood and distance (d) to the mother tree. An exponential function was used to relate ϕ to d (Figure 1). When the distance parameter β is 0, ϕ is unrelated to d, and as β increases in value, near neighbors are increasingly favored in mating. The mating model with three parameters, s, m and β, was applied to the combined offspring data of all five ramets (total = 173), giving maximum likelihood estimates of s = 0.00 (SE=.03), m = 0.69(.06) and β = 0.02(.07). Only $\hat{m}$ was significantly (P<.05) different from zero. We conclude from these results that most pollen effective in fertilizing viable offspring came from outside the neighborhoods, that the proportion of viable selfed offspring is low (or zero), and mating success within the neighborhoods is unrelated to distance between mates. The latter result is consistent with earlier seed orchard studies in Douglas-fir (Erickson and Adams 1989) and Scots pine (Shen et al. 1981) using the rare marker approach. These studies indicated that the large pollen fecundity and phenology differences among orchard clones can overwhelm distance as an important determinant of mating success within 20 to 30 m of mother trees; however, when floral synchrony between males and females is high, near neighbors are favored.

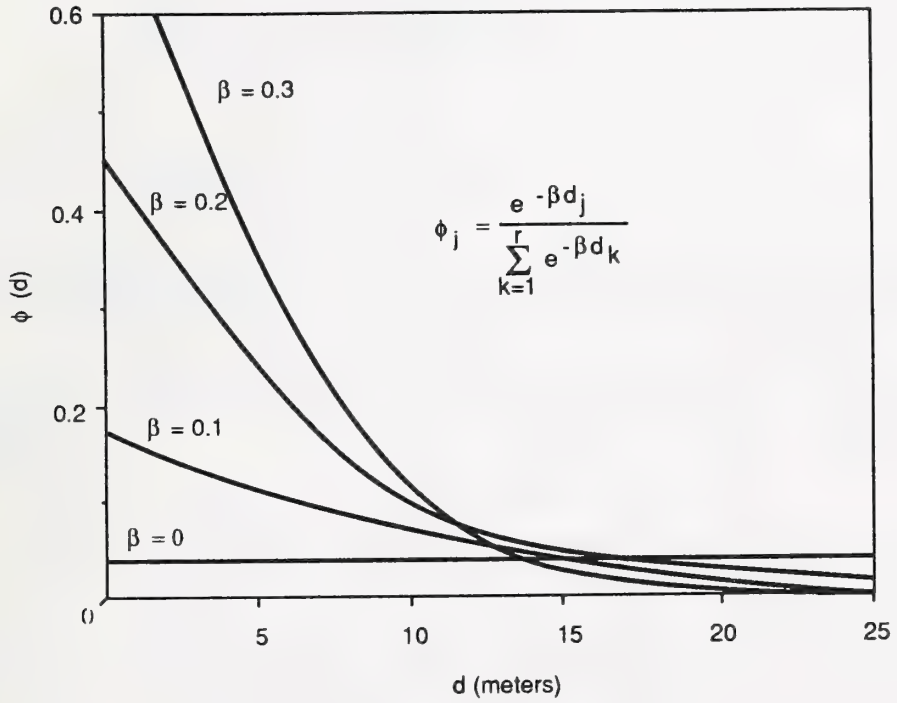

Figure 1. Relationship between mating success of a male (ϕ) and distance to the mother tree (d) for various values of the distance parameter (β). Also given is the formula for computing ϕ for the jth male (ϕ_j), where d_j is the distance the jth male is to the mother tree and r is the number of males in the neighborhood.

To investigate the relationship of pollen fecundity and phenology of males to mating success within the neighborhoods, we applied a second model. Both flowering factors were combined into a single measure called effective pollen fecundity (f), which was calculated for each male as its relative pollen production multiplied by the degree to which its pollen shedding period overlapped with the female receptivity period of the mother tree. Estimates of relative pollen production and the flowering periods of each ramet in the study block were derived from observations on flowering in the spring previous to seed collection (Adams, unpublished). The number of ramets per clone in the orchard block ranged from 2 to 29, and the proportion of the pollen crop contributed by each clone ranged from 0.41 to 10.0%. Fifty percent of the clones produced 80% of the pollen crop. The mean date at which each of the clones initiated pollen shedding ranged over two-weeks, although 75% of the mean initiation dates occurred within a five-day period.

As for the distance relationship, we used an exponential function to relate ϕ to f (Figure 2). When the effective fecundity parameter γ is 0, ϕ is unrelated to f, but as γ increases, ϕ increasingly becomes a function of f. Since s was found not to differ significantly from zero in the previous analysis, the mating model with two parameters, m and γ, was applied to the combined offspring data of the five ramets. The maximum likelihood estimates of both parameters, $\hat{m} = 0.68(.06)$ and $\hat{\gamma} = 0.34(.15)$, were significantly different from zero. As with the previous model, migrant pollen from outside the neighborhoods accounted for nearly 70% of the viable offspring of the mother trees. In addition, there appears to be at least a weak relationship between relative mating success of males within the neighborhoods and f. Thus, effective pollen fecundity is more useful for explaining mating success differences among males in the neighborhoods, than is distance to mother trees.

Because distance to the mother tree and effective pollen fecundity appeared to interact in their influence on mating success in earlier seed orchard studies based on rare markers (Erickson and Adams 1989, Shen et al. 1981) we tested a third model, which in addition to m and γ, included a parameter specifying the interaction between d and f. The fit of this model to the combined offspring data was not significantly better than the two-parameter (m and γ) model.

The greater γ is, the more skewed will mating success be towards fewer males with higher f, and the less will be the genetic diversity present in pollen gametes produced by outcross males within the neighborhoods. Since ϕ measures the proportional contribution of an individual to the outcross pollen pool within a neighborhood, the influence of differences in f on genetic diversity can be evaluated by examining the variation of $\hat{\phi}$ among males in the neighborhood, where $\hat{\phi}$ is calculated using the exponential function (Figure 2) and γ is substituted for γ. For each neighborhood, a ϕ for each clone was obtained by summing the $\hat{\phi}$ values of all ramets of the clone that were present. On average, each neighborhood included 15.4 (range 13 to 18) clones. If each clone in a neighborhood contributed equally to the

outcross pollen pool, $\hat{\phi}$ would be the same for all clones. In reality, $\hat{\phi}$ may range as much as from 0.01 to 0.26 in any one neighborhood; nevertheless, there were only 4 cases where $\hat{\phi}$ exceeded 0.15. Thus, the outcross pollen gametes produced by males within the neighborhoods, were not dominated by one, or a few, clones.

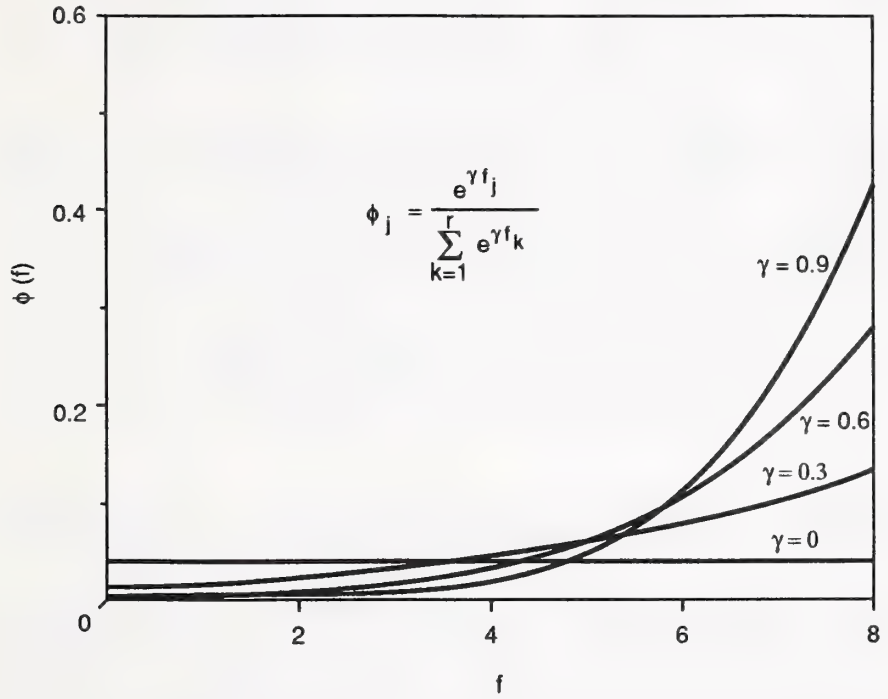

Figure 2. Relationship between mating success of a male (ϕ) and its effective pollen fecundity (f) for various values of the effective fecundity parameter (γ). Also given is the formula for computing ϕ for the jth male (ϕ_j), where f_j is the effective pollen fecundity of the jth male and r is the number of males in the neighborhood.

The effective number of outcross male clones in a neighborhood (N_e) is the theoretical number of males necessary to generate an equivalent amount of genetic diversity in the outcross pollen pool as produced by the actual males in the neighborhood, given that all males in the neighborhood have an equal chance of mating with the mother tree. N_e can be calculated as $1 / \sum \hat{\phi}_j^2$, where $\hat{\phi}_j$ is the estimated mating success of the jth clone, and the summation is over the total number of clones in the neighborhood (Crow and Kimura 1970). N_e averaged 10.1 over the 5 mother trees (range 8.1-12.8), indicating that despite differences among clones in effective pollen fecundity, considerable cross-fertilization occurred within the neighborhoods.

CONCLUSIONS

Although research on mating patterns in seed orchards has accelerated in recent years with the availability of isozyme genetic markers, most of

this work has been directed at estimating proportions of progeny due to selfing. On average, this proportion appears to be low in clonal orchards, but occasionally individual clones have high levels of viable selfed offspring. These clones need to be identified and either rogued from the orchard, or treated by pollen management techniques (e.g., supplemental mass pollination) to reduce the amount of self-fertilization to acceptable levels (El-Kassaby and Ritland 1986, Webber 1987).

In the future, research on the outcrossing component of the breeding system needs to be emphasized since there is little known about this aspect of mating. The high levels of pollen contamination that have been observed in several orchards is particularly disturbing because of the severe negative impact it has on genetic quality of seed crops. We need a better understanding of how various parameters such as orchard size, age, composition, and degree of isolation influence levels of contamination; and the degree to which pollen management techniques are effective in limiting contamination (El-Kassaby and Ritland 1986, Wheeler and Jech 1986). If wind-pollinated seed orchards are to be relied upon in the future, satisfactory solutions to the pollen contamination problem must be realized.

The neighborhood model seems to be promising for studying patterns of cross-fertilization. Estimates of mating parameters in a Douglas-fir orchard block based on this model were consistent with previous information on cross-fertilization in seed orchards. In particular, for any one mother tree ramet, crosses with near neighbors appear to account for only a small percentage of the total offspring. In addition, the data suggest that with low pollen contamination, cross-fertilization among clones may be extensive. These conclusions, however, are based on only a few studies and must be considered tentative. In addition to the need for better information on cross-fertilization in orchards not subjected to pollen management, the influence of practices such as flower stimulation and bloom delay on cross-fertilization patterns must be understood. This information is necessary if the influence of orchard designs and management regimes on the genetic efficiency of seed crops is to be adequately assessed (Adams 1983).

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GENETICS OF DOUGLAS-FIR SEED ORCHARDS: EXPECTATIONS AND REALITIES

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Abstract.--This paper summarizes the results of a decade of research conducted on Douglas-fir seed orchards in British Columbia, Canada. Firstly, the seed orchard's basic assumptions (i.e., reproductive synchrony, parental balance, minimal inbreeding, isolation, and random mating) were systematically examined in clonal and seedling orchards. Secondly, the impact of two crop management techniques, supplemental-mass-pollination and overhead cooling, on the genetic efficiency (quality and quantity) of orchards were assessed in a controlled designed experiment. Thirdly, several management practices that are commonly used in British Columbia were evaluated. These include the reliance on cone count as a sole criterion for determining parental balance, the management of crop trees only, the removal of non- or low-seed cone producing trees, and the normally accepted loss of some seeds from a few trees as a prerequisite to cone harvesting. Finally, a novel approach to genetic thinning that optimizes both genetic gain and diversity as well as maintaining flexibility is proposed.

INTRODUCTION

Seed orchards represent the link between breeding programs and reforestation activities through the consistent delivery of abundant crops of genetically improved seed to meet a predetermined need. The attainment of genetic gain is dependent upon maintaining the same frequency of desirable genes in the orchard seed crops at the same level that is present in the selected population. The maintenance of similar allele frequencies between the seed orchard population and its seed crops requires attainment of the random-mating assumption of the Hardy-Weinberg theorem.

Random mating in seed orchards (i.e., panmictic equilibrium) can only be achieved if the genetic entities (i.e., clones or

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families) are in reproductive synchrony and have similar reproductive output (i.e., parental balance). In addition, since coniferous forest tree species are mainly wind-pollinated and often display strong inbreeding depression, the potential of pollen migration (i.e., contamination) from extraneous undesirable sources and inbreeding through self-fertilization and/or consanguineous mating is of concern. Therefore, a seed orchards genetic efficiency, defined as the degree to which the orchard crop reflects the genetic superiority and diversity present in the orchard population, is dependent upon maximizing reproductive synchrony and output equality and minimizing inbreeding and contamination. In short, a seed orchard must represent a nearly perfect, closed, panmictic population.

Due to their importance, seed orchards and their genetics are the focus of increased research activities. Their robustness to violation of the above-mentioned conditions has been assessed for several species. It is not surprising that most of these conditions are not fully met and that several management options were proposed or implemented. Supplemental-mass-pollination (SMP), the broadcast application of viable pollen to non-isolated receptive strobili (Wakeley et al. 1966), is the most common crop-management method used by seed orchard managers. Overhead cooling of orchard trees, another crop-management practice, was proposed as a means of reducing orchard contamination by delaying reproductive bud development relative to background pollen sources (Silen and Keane 1969). Although its use is restricted to the Pacific Northwest, the cooling treatment is gaining increased attention as a viable method for reducing pollen contamination.

This paper summarizes the results of research conducted in Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seed orchards which systematically examined basic seed orchard assumptions and their inter-relationships. In addition, the impact of SMP and overhead cooling methods on the genetic efficiency of seed orchards is evaluated.

REVIEW OF DOUGLAS-FIR SEED ORCHARD GENETIC RESEARCH

Reproductive Phenology

Synchronization of the phenology of reproductive strobili among different clones or families is a fundamental need for any successful seed orchard operation. Failure of synchronization affects other, if not all, assumptions. It is well known that both time and temperature (summarized as heat sums) have a significant effects on flowering time (Kramer and Kozlowski 1979). Since seed orchards are established with clones or selections that may require different heat sums, it is to be expected that time differences of reproductive phenology will be found.

Reproductive bud phenology information for every sexually active tree in a high-elevation Douglas-fir seed orchard was collected for six years. Each tree was monitored on alternate days throughout the pollination season. The critical stage of seed-cone receptivity and pollen shedding was recorded following the classification proposed by Ho (1980) and Owens et al. (1981). In addition, background pollen was monitored using pollen traps (for more details see Fashler and Devitt 1980). The six year study encompassed three years when the flowers opened under natural conditions and another three years under overhead cooling. Differences were found in both the length of pollination period and the number of genotypes reproductively active at a given time (Fashler and El-Kassaby 1987). Figure 1 presents a typical pattern of the reproductive phenology and timing in the seed orchard versus background pollen under both treatments. When the seed orchard was not cooled, the female receptivity and pollen release periods averaged 24.3 and 27.7 days, respectively. Under cooling, the pollination period was reduced by an average of seven days to 17.3 days for females and 18.3 for males. In addition, the maximal number of flowering females and male occurred later than the local pollen flight and developed in a relatively shorter time in the cooled years compared to years under natural conditions (Fig. 1).

Differences in the number of receptive females and pollen-shedding males were apparent at various stages (i.e., early, intermediate and late) in the pollination period in both cooled and uncooled years (Fig. 1). Cooling appears to have reduced greatly the magnitude of these differences. The disproportion in females and males suggests potential differences in the filled seed produced in each phenological class due to lack of pollen and/or availability of related pollen (El-Kassaby et al. 1984; Woods and Heaman 1989).

The length of pollination period and its differentiation into early, intermediate and late reproductive phenology classes suggest that a major deviation from panmictic equilibrium exists. Since females are receptive for only four to six days and the optimal duration of pollen shedding varies between three to five days, mating between early- and late-flowering trees is not possible. Then the effective breeding population size is reduced and a continum of small breeding populations throughout the pollination season will be the result, as opposed to one panmictic population.

Reproductive phenological differences can affect all seed-orchard assumptions in several ways. Under panmixia, the expected selfing rate is equal n/n^2, where n is the number of clones. Therefore, in a hypothetical seed orchard that consists of 30 clones, the expected selfing rate is 3.33%. If this orchard is divided into three non-overlapping breeding sub-populations with equal proportions (1:1:1) or unequal proportions (1:4:1) of clones. The expected selfing rates for these situations will be 10 and 6.67%, respectively. This

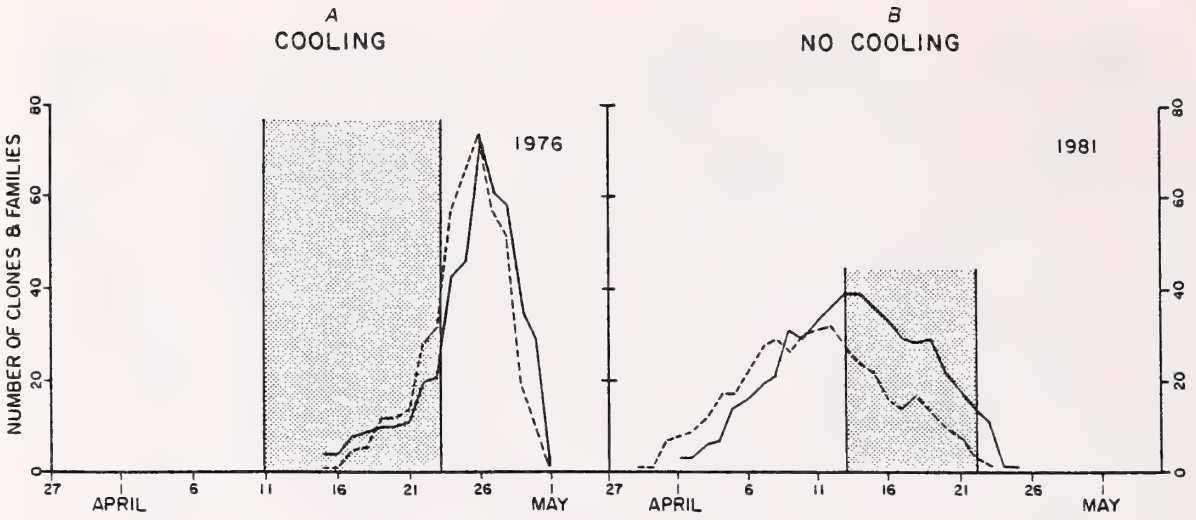


Fig. 1. Reproductive phenology comparison between (A) cooled (1976) and (B) uncooled (1981) years indicating the timing of the local background pollen flights.

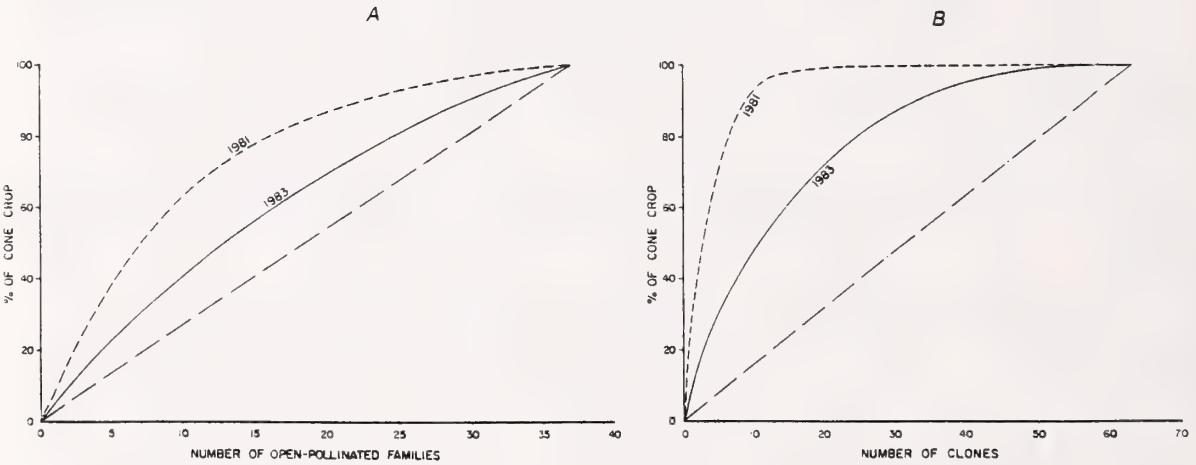


Fig. 2. Cumulative seed-cone production curves for open-pollinated families (A) and clones (B) for a good (1983) and a poor (1981) cone year. Straight line represents equal contribution.

hypothetical orchard produced three- and two-fold differences in selfing expected when panmictic disequilibrium existed.

The frequency and timing of receptive females and pollen-shedding males also affects the rate of pollen contamination. If the background pollen is high in early or late phenology classes, it is expected that most of the receptive sites will be saturated by background unrelated pollen (El-Kassaby and Ritland 1986a).

Reproductive phenological differences also affect the amount of gametic contribution by every clone to the resultant seed crop. A high seed or pollen producing clone in the early or late reproductive phenology classes could contribute the same as or less than the amount contributed by a low or medium reproductive output clone during the height of pollination (El-Kassaby and Askew 1989).

Variation in reproductive developmental phases observed in one Douglas-fir seed orchard was not associated with variation in the timing of seed maturation, therefore, cone harvesting can begin irrespective of flowering phenology, before cone opening starts (Edwards and El-Kassaby 1988). The results from the flowering phenology and date-of-cone-collection study indicated that seeds in all phenological classes had matured by the time they were harvested, and that seed-ripening mechanisms have adapted to reach completion over different lengths of time (Edwards and El-Kassaby 1988).

Reproductive Output

The maintenance of similar allelic frequencies between a seed orchard and its seed crop is dependent upon the presence of balanced gametic production among the orchard's genetic entities. Due to the ease of estimating clonal seed-cone production, seed orchards' parental balance often are based solely on seed-parent contributions. In fact, the so-called "20/80" rule (i.e., 20% of the clones produce 80% of the cone crop) was coined by the North Carolina State Tree Improvement Co-operative in 1976 (Anon. 1976). Since then, parental balance values based on seed-parent contributions have become an accepted method to evaluate and even to provide genetic rating of seed orchard crops.

i) Parental balance based on seed-cone production

In an attempt to estimate the parental balance of a clonal/seedling Douglas-fir seed orchard, individual tree cone-counts were conducted at harvest over the 1976-1983 period. Variation in cone production among families and clones over the eight-year-period revealed the following: 1) seedling trees' cone crop consistently exceeded that of clonal trees despite physiological maturity differences, 2) a biennial cone-bearing trend was apparent, notwithstanding an overall increase in

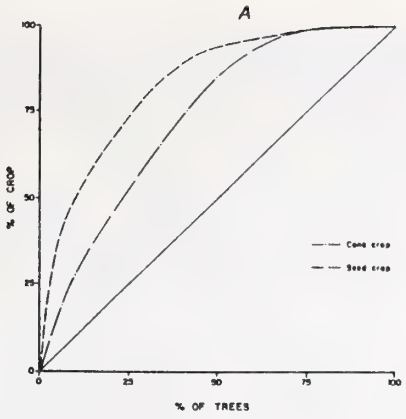
production with age, 3) clones and families were classified as consistently high, consistently low or inconsistent cone producers, suggesting that both genetic and environmental factors influence cone production, and 4) parental balance was largely affected by the crop size and genetic classes (Fig. 2a,b).

Parental balance of clones and families was assessed by cumulative cone-yield curves following the method of Griffin (1982). Parental contribution varied substantially among years and between genetic classes (Fig. 2a,b). In a good cone year (1983), approximately 50% of the families produced 63% of the total cone crop, a near-ideal situation. However, in a poor cone year (1981), the top 50% of the families produced 83% of the total crop, with the remaining 50% producing only 17% of the total crop (Fig. 2a). The clonal trees' production was more distorted than that of families in both good and poor cone years (Fig. 2b). In 1981, 10% of the clones produced 80% of the crop and in 1983, 50% of the clones produced 88% of the crop total as compared to 63% for families.

Various crop-management options have been proposed to adjust for the observed imbalance in cone crops. These include SMP (El-Kassaby et al. 1986a, 1989c; El-Kassaby and Reynolds 1989; Reynolds and El-Kassaby 1989), mixing seed crops from several years (Varnell et al. 1967; Schmidtling 1983; El-Kassaby et al. 1986a, 1989c), abortion of small cone crops (El-Kassaby et al. 1986a; El-Kassaby and Ritland 1986a), individual-clone harvesting and seed extraction (Reynolds and El-Kassaby 1989), and selective use of cone-induction methods (El-Kassaby et al. 1989c).

ii) Parental Balance Based on Seed Production

The preceding section on parental balance assumed that reproductive energy is equal to reproductive success (i.e., cone size and the number of filled seeds per cone is equal across the different parental groups). A comparison of parental balance among 30 Douglas-fir trees based on seed-cone crop to that of filled-seed crop (Reynolds and El-Kassaby 1989) demonstrated that the seed-crop data have provided a more-accurate assessment of the genetic diversity and the family representation in the resultant seed crop than did the cone crop (Fig. 3a). Parental balances of "57/90" and "40/90" were obtained for seed-cone and seed crops, respectively (Fig. 3a). In addition, the reproductive-rank order of the studied trees changed substantially when seed production was used (Fig. 3b). Five out of the top 10 cone-producing trees remained among the top 10 seed producers, while the other five dropped to "intermediate" seed production (rank 11-20). The change in rank among the 10 intermediate cone producers indicated the presence of high, intermediate and low seed producers. On the other hand, nine out of the 10 "low" cone producers maintained their relative rank as seed producers. Comparing the results of parental



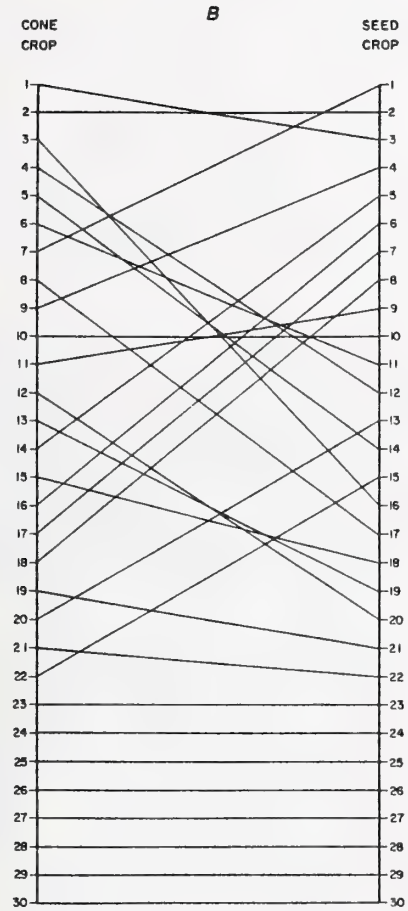
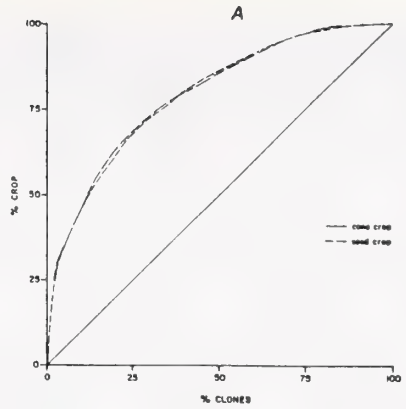


Fig. 3. (A) Cumulative cone and seed production curves for 30 Douglas-fir trees. (B) Rank order presentation for the same 30 Douglas-fir trees, based on cone and seed crops.



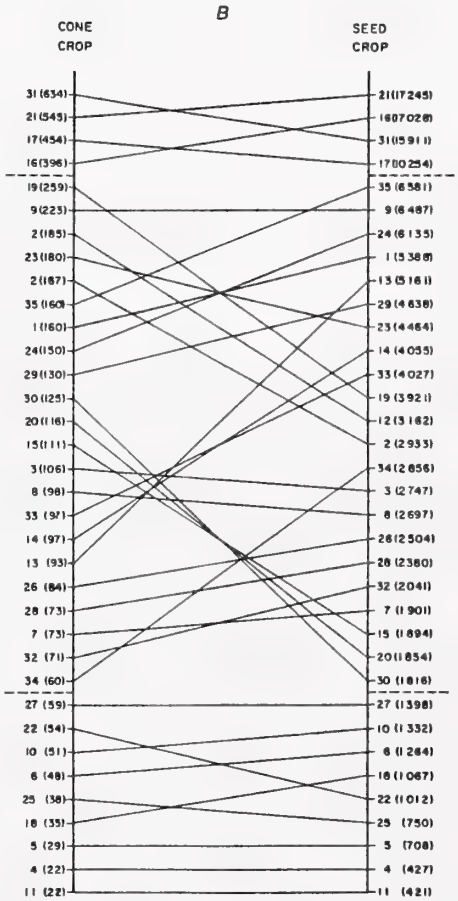


Fig. 4 (A) Cumulative cone and seed production curves for a 1.5-generation Douglas-fir seed orchard. (B) Rank order presentation of the orchard clones based on cone and seed crops.

balance based on the two approaches indicates the following: 1) the estimate based on cone harvest did not reflect the distortion in the seed crop, 2) 90% of the cone crop was obtained from 17 trees (57%), while 90% of the seed crop was obtained from 12 trees (40%) (smaller genetic base), 3) the tree that ranked number 8 in seed production was among those producing the 90% of the seed crop, but it was not among those producing 90% of the cone crop (different genetic representation) and 4) there was little rank change in the "low" cone and seed producers.

Table 1. Variation in the number of cones (C) and filled seeds (S) per ramet for the studied 35 Douglas-fir clones combined and for the three production classes (high, intermediate and low) (values are percentage of the total sum of squares).

Source of Variation	Combined			High			Intermediate			Low		
	d.f.	C	S	d.f.	C	S	d.f.	C	S	d.f.	C	S
Among Clones	34	48.2**	49.3**	3	4.6ns	6.0ns	21	11.0ns	14.5ns	8	3.3ns	5.8ns
Within Clones	166	51.8	50.7	21	95.4	94.0	98	89.0	85.5	47	96.7	94.2

**, $P<0.01$; ns, not significant at $P<0.05$

The results obtained from this study clearly demonstrate the difference between cone versus seed crop use in estimating parental balance. However, it failed to demonstrate the within-family or clone variation in order to determine how much of the predicted variability in performance could disappear with averaging when an entire orchard data set is considered. To address this question an additional study was conducted on a 1.5-generation Douglas-fir orchard and the within- and among-clonal variation in cone and seed crops were evaluated (El-Kassaby and Reynolds, unpublished). A similar trend was observed and changes in clonal cone and seed rank were obtained (Fig. 4a,b), but the parental-balance curves appeared to be identical (Fig. 4a). It must be emphasized that the clonal representation at any point on the two identical cone and seed yield curves is different (see Fig. 4b). Although the rate of distortion was the same in both cases, the genetic representation was different. The within-clone variation accounted for approximately 50% of the total sum of squares for both cone and seed analyses (Table 1). However, when the clones were grouped into three classes of production level (high, intermediate and low), the within-clone variation accounted for 85-97% of the total variation (Fig. 4b and Table 1). The separation of clones into three reproductive classes was based on the non-overlapping nature of clonal rank in both cone and seed production (Fig. 4b). In summary, this study demonstrated the following: 1) the presence of high within-clone variation in cone and seed production, 2) parental balance, seemingly

identical for cone and seed curves, represent different genetic contributions for cones and seed at any given point and 3) the clones' reproductive energy differed from their reproductive success.

iii) Cost of Reproduction

It has become common practice to select orchard trees with consistently high cone production for inclusion or retention during operational thinning. This makes sense from the point of view of the seed orchard manager since it should facilitate future seed production (Danbury 1971). There is a growing body of theoretical literature, however, that casts doubt on the validity of this procedure. It is known by various names, but most commonly is called the theory of life history evolution. The theory maintains that since there are finite resources available to an organism, selection that involves increasing the resource allocation to one trait will of necessity decrease resource availability elsewhere. More specifically, selection for higher reproductive output should concomitantly select for slower growth. Stearns (1977,1980) has presented mounting empirical and experimental evidence that demonstrate such trade-offs when selection is imposed on a population. A study in which 8-year cone production and growth increments (1976-1983), based on seed-cone count and annual ring width, was conducted to address this question (Barclay and El-Kassaby 1988; El-Kassaby and Barclay 1989). The population studied consisted of 29 open-pollinated (half-sib) Douglas-fir families that are growing in a seed orchard. The orchard environment is highly controlled and environmental influences were minimized, making the underlying genetic patterns more readily detectable. The presence of family structure permitted the estimation of phenotypic, genetic and environmental correlations using the analysis of variance and covariance methods specific to half-sib families (Becker 1984).

Table 2. Genetic correlations (r_g) between seed cone count and annual ring increment for 29 Douglas-fir open-pollinated families for 1976-83.

Year	1976	1977	1978	1979	1980	1981	1982	1983
r_g	-0.776	-0.412	-0.586	0.181	-0.199	-0.316	-0.265	0.213
Cone Yield	324,105	21,473	7,186	50,077	75,588	51,699	8,541	33,787

Six out of the eight genetic correlations were negative (Table 2) reflecting different genetic propensities among families to either allocate their resources primarily to cone production or to growth. These negative correlations imply the existence of genetic variability in the proportional allocation of photosynthate to reproduction and growth, and that selection

for cone production will result in negative selection for growth. In conclusion, this study demonstrated the presence of a tradeoff between reproduction and growth. Thus, the practice of selecting seed-orchard clones for high cone production or managing "cone crop" trees only in seed orchards may be expected to yield slower-growing progeny.

Relation Between Reproductive Phenology and Reproductive Output

An orchard's parental balance is often based solely on seed-parent contribution, with either an implicit or explicit assumption that the pollen contributions are balanced or that they are distributed in similar proportions to the seed-parent contributions (i.e., high, positive and significant correlation between male and female reproductive energy and success is present). Such an assumption places too little emphasis on 50% of the gametic pool (i.e., male pool) and the differential viability and selection among gametic sources. It should be emphasized that the gametic contribution by high or low pollen-producing clones or families is influenced by their reproductive timing in relation to other clones or families in the seed orchard. High pollen-producing clones during the early or late reproductive period could have similar gametic contributions to "low" clones during the height of pollination. Therefore, evaluation of the reproductive phenology and output level, for both male and female strobili, concurrently over the entire orchard population will provide a more realistic assessment of individual clones' contribution to the gametic pool.

The relationship between reproductive phenology and output in determining the gametic contribution by family was studied for 97 full-sib families in a 15-year-old Douglas-fir seed orchard (El-Kassaby and Askew 1989). Individual family gametic contribution was determined after considering: 1) the time of seed-cone receptivity, 2) time of pollen shed, 3) the level of seed-cone production, 4) the level of pollen production and 5) the reproductive phenology overlap between each family and all other families in the orchard. The study demonstrated that any evaluation based on reproductive phenology (Fig. 5a) and parental balance curves (Fig. 5b) alone is limited and that their concurrent use is more informative in obtaining an accurate assessment of each family's potential gametic contribution to the orchard crop. The study indicated that some of the top seed or pollen producers' gametic contributions were surpassed by lower ones when reproductive phenology was considered. The method presented could be used also as a management tool to direct SMP and/or cone-induction activities towards the low gametic contributors to adjust parental gametic balance toward equality.

Pollen Contamination

The maintenance of similar allelic and genotypic frequencies between a seed-orchard population and its seed crop is dependent

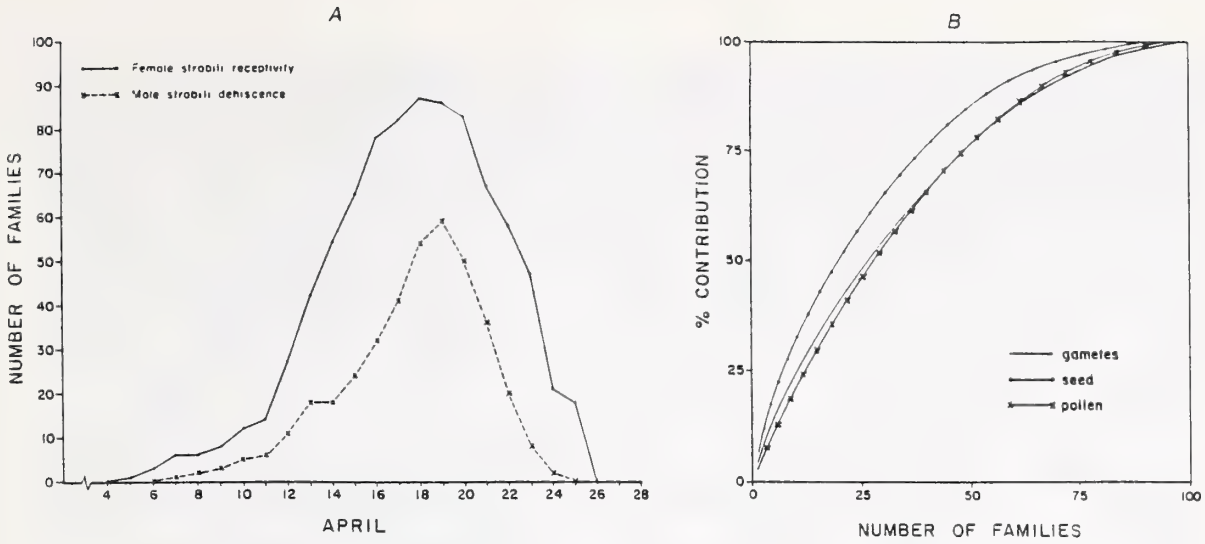


Fig. 5 (A) Reproductive phenology for a 15-year-old Douglas-fir seedling seed orchard. (B) Estimates of cumulative seed and pollen cone and gamete contributions for seed crop from 5 (A).

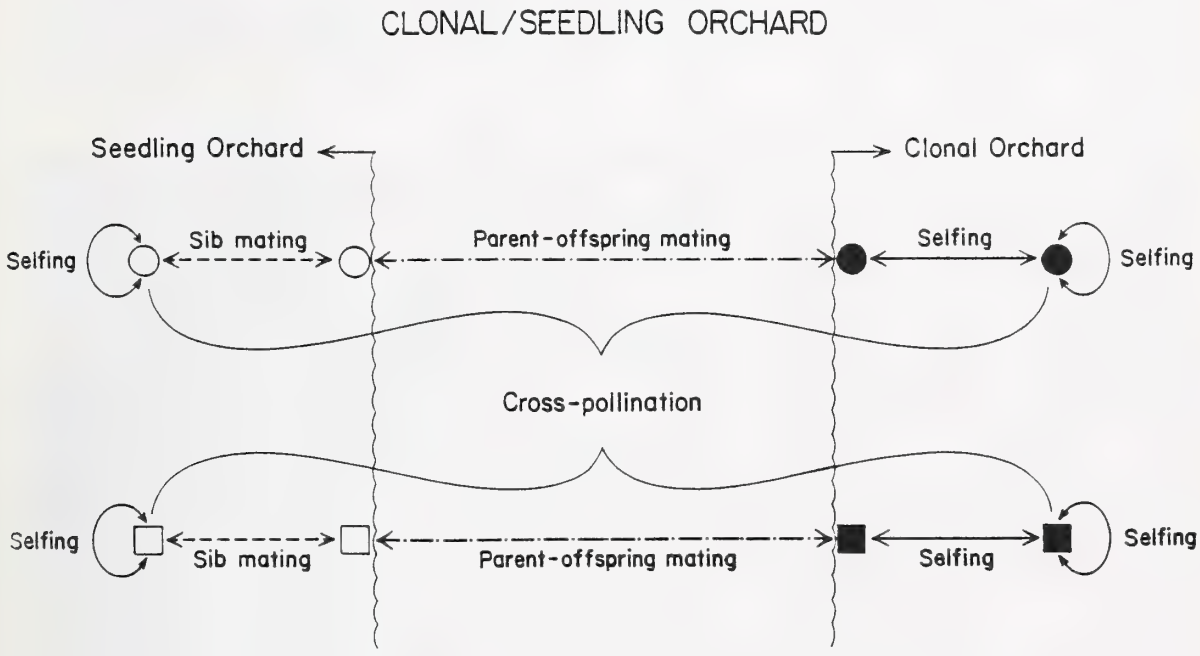


Fig. 6 Diagramatic representation of the possible mating types in clonal, seedling, and clonal/seedling orchards. ● and ■, ramets of specific clones (representing ortets ● and ■ respectively); ○ and □, open-pollinated seedlings from ortets ● and ■ respectively; ←→, selfing; ←--→, sib mating; ←—→, parent-offspring mating; ⌒, cross-pollination.

upon the fulfillment of the random-mating assumption of the Hardy-Weinberg theorem in the absence of selection, mutation and migration. Pollen migration (gene flow) provides a mechanism for maintenance of genetic variability, particularly in sessile, wind-pollinated, long-lived organisms like forest trees. The amount of gene flow (i.e., contamination) from surrounding unselected trees into seed orchards affects the genetic quality of seedlots in the following ways: 1) reduction of the expected genetic gain, 2) reduction of the within-orchard outcrossing rate, 3) production of maladapted stock and 4) change in the allelic and genotypic frequencies between the seed orchard population and its seedlots.

Methods used to reduce pollen contamination in seed orchards include: 1) geographical isolation by establishing orchards outside the species' range (Sarvas 1970; Hadders 1972) or at different elevations (Silen 1963), 2) reducing the frequency of outside pollen by the use of SMP (Woessner and Franklin 1973; Bridgwater and Trew 1981; El-Kassaby and Ritland 1986a), establishing larger orchards (Wright 1953), and surrounding the orchards by isolation zones (Wright 1953; Squillace 1967), or 3) physiological (i.e., temporal) isolation of the orchard through phenological manipulation by the use of water-spray cooling treatment (Silen and Keane 1969; Fashler and Devitt 1980; El-Kassaby and Ritland 1986b; El-Kassaby and Davidson 1989a).

The level of pollen contamination is affected by the proximity to and the size of the contamination source, reproductive synchrony between the orchard and outside source, crop size, seed orchard pollen crop size and the crop management practices used. Contamination estimates of 40 and 52% have been reported for two Douglas-fir orchards from Oregon (Smith and Adams 1983) and 44% for an orchard form Washington (Wheeler and Jach 1986). Contamination levels of 24% for early- and 18% for late-flowering trees for a Douglas-fir seed orchard from British Columbia when the cooling treatment was not used (El-Kassaby and Ritland 1986a) and a 9% for early-flowering trees when the cooling was used have been found (El-Kassaby et al. 1986a). The overall contamination level estimated for that orchard when the cooling treatment was used reached a record low of 0.2±5.7% (El-Kassaby and Ritland 1986b). The use of SMP as a contamination-prevention method proved effective. No contamination was detected for intermediate-flowering trees that received SMP (El-Kassaby and Ritland 1986a) or for orchard blocks receiving SMP alone or in conjunction with cooling (El-Kassaby and Davidson 1989a).

Inbreeding

The mating system, the union of male and female gametes and their genetic relationship, plays a crucial part in determining subsequent population structure and on the way genetic information is being transmitted and distributed from one

generation to another. Although estimates of natural outcrossing in conifers have been found to be quite high (t>0.9; Adams and Birkes 1988, for review), the level of inbreeding in seed orchards, even if it is low, is important since most forestry programs rely on non-competitive plantings in both nursery production and plantation establishment phases.

The outcrossing rates of several plant species have revealed marked differences under different environmental conditions (Clegg 1980). Orchard population structure (clonal vs. seedlings) (Ritland and El-Kassaby 1985), density (El-Kassaby *et al.* 1986b), phenological differences (El-Kassaby *et al.* 1988), contamination level (El-Kassaby and Ritland 1986a; El-Kassaby *et al.* 1989d) and reproductive output (Denti and Schoen 1988) all have affected outcrossing rate significantly.

In general, outcrossing-rate estimates obtained from seed orchards are higher than those reported for natural stands of the same species. This suggests that population structure (i.e., the physical arrangement of related and unrelated individuals within a population) affects the rate of outcrossing. If orchard population arrangement can increase the rate of outcrossing, then any additional manipulation of the pollination ecology could also further reduce inbreeding and, consequently, improve the seed orchard's genetic efficiency.

Cone position in the crown can influence inbreeding; higher levels of inbreeding in lower crowns of Douglas-fir orchards were reported (Shaw and Allard 1982; Omi and Adams 1986; El-Kassaby and Davidson 1989b). However, when the overhead cooling treatment was applied, non-significant differences were reported (El-Kassaby *et al.* 1986b; El-Kassaby and Davidson 1989b). In addition, the overall outcrossing rate obtained from cooled orchards was higher than that obtained from uncooled ones, even under a shorter pollination season (El-Kassaby and Davidson 1989b). The shorter pollination season produced a pollen cloud of related and unrelated pollen, and early zygotic selection acted as a mechanism to weed out inbreds. In general, the clonal orchards' inbreeding level was lower than seedling ones due to the presence of only one type of inbreeding (i.e., selfing) as opposed to selfing and sib-mating in seedling orchards and parent-offspring mating of clonal/seedling orchards (Fig. 6). Reproductive phenology classes also produced different inbreeding levels: early- and late-flowering trees produced lower proportions of outcrossing than trees with more intermediate flowering periods (El-Kassaby *et al.* 1988).

Impact of Crop-Management Practices on the Quality and Quantity of Seed Crop

The impact of SMP and overhead cooling on the joint levels of contamination and outcrossing, and seed yield, was assessed in a 13-year-old seedling Douglas-fir seed orchard. A 2x2 factorial arrangement of SMP/no SMP and cooling/no cooling was

applied to four identical blocks of trees (El-Kassaby and Davidson 1989a). The four treatment combinations studied were spatially and temporally isolated by buffer blocks and a 9-day reproductive bud phenology delay. The rate of outcrossing and contamination in the seed crop was determined from a sample of 325 trees (79-85 trees/block) that provided a total of 5,964 viable seeds (1441-1574 seeds/block). The study revealed the following conclusions: 1) no contamination was obtained when either cooling or SMP was used, 2) no inbreeding was observed when cooling and SMP were used concurrently, and 3) SMP was effective in reducing consanguineous mating, but not to the level attained form cooling and SMP combined (Table 3).

Table 3. Joint levels of outcrossing and contamination for all cooling/no cooling and SMP/no SMP treatment combinations for four identical 13-year-old Douglas-fir orchard blocks.

Treatment	Cooled		Uncooled	
	SMP	No SMP	SMP	No SMP
Outcrossing (t)	1.090±0.002	0.978±0.018*	0.970±0.019*	0.970±0.019*
Contamination 1/	-0.110±0.357	-0.121±0.428	-0.016±0.337	0.005±0.267

* Rejection of the null hypothesis that $t = 1.00$ at $P<0.05$.
1/ Negative values indicate lack of contamination.

Seed-yield comparisons were determined form a sample of 50 cones form each of 20 trees in each treatment combination. Potential seed yield per cone, the average number of successful fertilizations and average number of filled seed per cone all showed no significant differences between cooling or SMP treatments and among cooling x SMP treatment combinations, indicated that the within-orchard pollen cloud was not a factor limiting seed yield. The average number of seeds infested by the Douglas-fir cone seed wasp (Megastigmus spermatrophus Wachtl) larva was significantly less when cooling was applied, indicating that the cooling treatment was effective in disrupting the synchrony between ovipositing females and developing cones (El-Kassaby et al. 1989a). A similar relationship was observed also by Miller (1983) for Douglas-fir cone gall midge (Contarinia oregonensis Foote).

The study indicted that the added benefits of SMP and/or cooling in mature Douglas-fir seed orchards are quality rather than quantity-oriented.

Genetic Gain Versus Genetic Diversity

Increasing the genetic gain in existing, tested, clonal, seed orchards could be accomplished by either genetic roguing or selective cone harvesting. Genetic roguing improves both seed and pollen parents due to the irreversible removal of low-

breeding-value clones form the orchard. On the other hand, selective cone harvesting improves only the seed parent since all clones (low and high breeding value) may act as pollen donors. Genetic roguing or selective cone harvesting are possible after ranking the orchard's clones according to their breeding value. Then the acceptance level (i.e., truncation point) is determined. In most cases however, the difference between the lowest accepted and the highest rejected clones is very small, while the difference between the highest and the lowest-accepted clones is very large. It is also worth mentioning that the ranking of clones is based on breeding values determined from progeny testing of material produced by controlled crosses and not from progeny collected from the orchard following wind-pollination. Therefore, genetic roguing may have the potential to eliminate clones that could be prime receptors of a major portion of the pollen being produced from the better clones and that do not substantially differ for accepted ones (i.e., high rejected and low accepted). In other words, although being conducted for the right reasons, genetic roguing could decrease the orchard gain rather than increasing it when specific combining ability is present.

Levels of genetic diversity between a rogued orchard's seed crop and a seed crop collected from a similar orchard following selective cone harvesting also are different. In an attempt to compare the levels of genetic gain and genetic diversity present in seed crops collected from a seed orchard following genetic roguing, selective cone harvest, and a combination of the two options, Lindgren and El-Kassaby (1989) have demonstrated on theoretical grounds that combining selective cone harvest with genetic roguing is the best option available because both genetic gain and diversity were optimized. Table 4 provides examples where the "relative effective clonal number" (equivalent to diversity) is constant (0.8) under the three options (Lindgren and El-Kassaby 1989). These are: 1) when 80% of the clones were harvested (P_H) after the removal of 20% (i.e., proportion remaining P_R = 80%) ($P_H = P_R = 0.8$), 2) when 60% of the clones were harvested after the removal of 10% of the orchard clones ($P_H = 0.6$ and $P_R = 0.9$) and leaving 30% to participate in pollination, and 3) when 50% of the clones were harvested and the remaining 50% were left to participate in the pollination ($P_H = 0.5$ and $P_R = 1.0$). The results indicate that the second option (i.e., combination between selective harvest form the best clones and removal of the lower ranked clones) provided the highest genetic gain (0.419) (equivalent to effective selection intensity that is proportional to genetic gain) compared to the removal of the low-ranking clones (0.350) or the selective harvest without any removal (0.399). It should be noted that the number of trees removed from the orchard affects both the rate of inbreeding and the contamination level. Both selective cone harvest and the combination of roguing and selective harvest options provide flexibility to adjust to the genetic composition of the orchard at advanced stages in the program.

Table 4. Comparison of genetic gain and genetic diversity (upper and lower numbers, respectively) for various fractions of clones harvested (P_H) and clones remaining (P_R) after genetic roguing (source: Lindgren and El-Kassaby 1989).

P_H \ P_R	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0
.1	1.755 .100	1.577 .160	1.457 .200	1.360 .226	1.276 .250	1.199 .267	1.126 .280	1.052 .291	0.976 .300	0.877 .308
.2		1.400 .200	1.279 .267	1.183 .320	1.099 .364	1.022 .400	0.948 .431	0.875 .457	0.797 .480	0.700 .500
.3			1.159 .300	1.062 .369	0.978 .429	0.901 .480	0.828 .525	0.754 .565	0.677 .600	0.579 .632
.4				0.966 .400	0.882 .471	0.805 .533	0.731 .589	0.658 .640	0.580 .686	0.483 .727
.5					0.798 .500	0.721 .571	0.647 .636	0.574 .696	0.496 .750	0.399 .800
.6						0.644 .600	0.570 .672	0.497 .738	0.419 .800	0.322 .857
.7							0.497 .700	0.423 .772	0.346 .840	0.248 .903
.8								0.350 .800	0.273 .873	0.175 .941
.9									0.195 .900	0.097 .973
1.0										0.000 1.000

Stress and the Generation of Heritable Variation

The life cycle of plants is characterized by the presence of two distinct phases: the haploid gametophyte, which produced gametes, and the diploid sporophyte, which contains cells capable of undergoing meiosis. Shoot apical meristems produce vegetative tissue until an external signal triggers a switch to floral development. Therefore, floral structures are derived from a set of cells which are part of the vegetative body of the plant. Annuals convert most of their apicals to floral

structures, while in perennials only a subset of the apices is converted from vegetative to floral development, leaving some to form vegetative buds which will support the following year's growth. The production of floral structures from vegetative parts provides a mechanism by which somatic mutations can be perpetuated through incorporation in the gene pool, perhaps increasing the overall variability of the species (Antolin and Strobeck 1985).

Stresses and shocks were considered among the major contributing factors in the appearance of these somatic variations (McClintock 1984). Durrant's (1958,1962) study on some varieties of flax is considered to be the classical example demonstrating the generation of somatic heritable variation under stressful environments (see Walbot and Cullis 1985, for review). The detection of these changed was easy in the flax study because the varieties used were stable, pure, inbred lines (i.e., maximum homozyosity and homogeneity). If shock or stress produces somatic mutation in outcrossing species, particularly in conifers, the detection of the mutation will be difficult due to the high heterozygosity and heterogenity characteristics of these species.

After working on the same flax varieties and many other plant species, Cullis (1987) indicates that the majority of the genomic alterations occur in some families of the highly repeated sequences of the DNA, and that higher plants have a genetically controlled variation-generating system that can be activated only by shocks. He stated also that the changes occur during the growth of the plants, when inducing conditions occur before meiosis, and that these changes can be passed onto the next generation(s). Thus, single plants may consist of a mosaic of genetically different parts. Therefore, the environment has a dual role in this case: on the one hand it is the agent causing variants to arise and on the other it acts to select among these variants.

The use of stress treatments, such as cultural modification (water stress, root-pruning, stem girdling, excessive nitrogen fertilization or starvation and high temperature), hormonal application (exogenous gibberellin) or their synergistic combinations, have been proven successful for flower promotion in seed orchards (see Pharis *et al.* 1987, for review). Conifers present a suitable material for the stress-triggered mutation mechanism due to the presence of a substantial amount of highly repetitive DNA sequences in their genomes (Dhillon 1987). Stress-induced mutations have not been reported for conifers but a few reports of subtle changes are published. Changes in germinative speed caused by nitrogenous fertilization (Allen 1961) and hormonal application (Puritch *et al.* 1979) were reported for Douglas-fir, and adverse heat-related effects on fertilization and seed development were observed for *Picea engelmannii* by Ross (1987). The use of clonal material will provide researchers with material analogous to the inbreed lines

used in the flax study and changes may be observed under controlled environments. The continual use of these practices without rigorous testing is dangerous and may effectively block further inquiry.

On the other hand, physiological manipulation of reproductive phenology development by overhead cooling in Douglas-fir seed orchards did not change the observed rate of abnormal embryos and germination parameters of seed crops under cooling compared to those developed under natural conditions (El-Kassaby et al. 1989b). It was concluded that the temporal delay of reproductive phenology caused by temperature manipulation as a result of the cooling treatment is within the species' biological limits.

CONCLUSIONS

The consistent supply of genetically improved seed for reforestation programs is dependent upon the fulfillment of several conditions in seed orchards. These include reproductive synchrony and reproductive equality among the orchards' genetic entities and minimizing both inbreeding and contamination levels. The proceeding review demonstrated that these conditions are: a) not fully met, b) inter-related, and c) substantially affected by the type of crop management practice used. The use of overhead cooling and/or SMP have proven to be effective crop management tools in reducing most deviations from panmixia. Recently, most of our crop management practices have been directed towards improving the genetic quality of seed crops since our production targets have been surpassed.

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Limitations of pollen management strategies for southern pine seed orchards

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Seed orchard pollen management can range from passive types such as careful arrangement of trees to encourage a uniform mixing of clones throughout the orchard to collecting pollen and artificially pollinating bagged female strobili. The general goals are the same for both extremes. Desirable crossing patterns are being encouraged and undesirable matings are being minimized.

In production orchards, estimation of the orchard's genetic potential is based on anticipated mating proportions. The assumption that all clones contribute equal pollen proportions to the seed crop is often used in wind-pollinated seed orchards. If pollen strobilus production varies among the orchard clones, a weighted average for the pollen contributions might be used. Also, production orchards typically include a buffer zone or pollen dilution zone which is used to minimize the proportion of matings that occur with non-selected or foreign pollen. The undesirable pollen may arise from trees of a like species with the orchard or from trees that will readily hybridize with the orchard species.

In breeding orchards, mating types and proportions are often managed by controlled pollination. That is, protecting the female strobili from unwanted pollen and then introducing a pollen source that has been selected for the specific mating. In both orchard types it may be desirable to augment standard pollen management practices with supplemental mass pollination (SMP) if unprotected strobili are being pollinated. Selected pollen may be mist blown throughout the orchard or applied directly to the strobilus. With all pollen management systems in all orchard types the goal is to focus the pollination process on specific desired matings.

Goals represent ideal situations. If all aspects of orchard management are exact and predictable, the goals will be met. However, as in any natural system, all practices are rarely exact and often unpredictable.

DEVIATIONS FROM AN IDEAL ORCHARD

A. POSITIONING

Establishing the geographic layout of a clonal seed orchard is usually a complicated task. First generation orchards may have as many as 150 clones with as many as 50 ramets per clone. Individual trees need to be placed in a pattern that will allow equal opportunity of cross-pollination among all clone pairs and minimum opportunity for selfing between ramets of the same clone. A minimum distance rule such as the 90-foot rule is often applied to the orchard. In this case, no two ramets of the same clone will be positioned any closer than 90 feet from each other. Placement of clones throughout the orchard may be a random process or a systematic process.

In the rare orchard where all trees produce equal amounts of male and female strobili and flowering is completely synchronized among all of the clones, a carefully constructed layout may allow for equal mating proportions among the different clones. If all ramets from a common clone are separated by sufficient distance, inbreeding may be effectively eliminated. However,

most orchards suffer from one or more deviations from the ideal situation.

Orchard roguing, graft incompatibility and other problems may cause the original placement scheme to be altered. As a result of these changes, some clones will have more ramets than others and the relative positioning will be far from the desired format.

Average distance between clones is not a good indicator of the potential for uniform pollen dissemination. Pollen flight probabilities usually follow an exponential distribution with increasing distance from the source tree. Tree pairs that are close together receive a disproportionately large share of the pollen than tree pairs separated by greater distances. Random positioning of trees should provide mathematically similar cross-pollination probabilities if sufficient numbers of ramets from each clone are used to minimize small sampling errors.

Systematic designs, in general, tend to group clones in similar patterns and severely limit interclone matings. However, a systematic design based on environmental factors and measured phenological and physiological characteristics of the trees may result in an ideal cross-pollination pattern.

B. FLOWERING PHENOLOGY

Flowering differences among clones occurs in all orchards and in some cases the variation is great enough to eliminate any possibility of equal or predictable representation among the clones. Variation in female strobilus production and male strobilus production on a given tree coupled with variation in times of receptivity and pollen flight may eliminate the possibility of desired mating types and severely skew the distribution of realized mating types away from a uniform distribution. Variation in female strobilus production is an inherent problem for pollen management in that it causes clonal differences in ability to receive pollen. A clone that is prolific in female strobilus production can certainly acquire more pollen than a clone with poor female strobilus productivity. Phenology variation coupled with placement problems can render genetic estimation of the seed crop almost impossible and at least destroy the precision and accuracy of the genetic estimates.

Disproportionate flowering or poor synchronization among clones is probably the largest single factor that affects the cross-pollination patterns in seed orchards. Problems discussed by Askew (1986), Blush (1986), El-Kassaby et al (1988), and others demonstrate the potential for complete exclusion of some clones from the pollen pool and the isolation of some seed parents from the orchard produced pollen. Using more ramets from low pollen production clones and reducing the representation of the high pollen production clones may help to alleviate the problem but the best tactic may be to carefully monitor the orchard phenology and to construct an orchard of synchronous clones as part of the roguing process.

Orchards with severe phenology problems need to have both the flowering characteristics as well as the seed production characteristics and genetic testing results incorporated into the selection process.

C. INSECT PREDATION

Insect predation of cones and seeds is another inherent problem that needs to be considered during pollen management development. Insect predation can eliminate as much as 90% of a trees cone crop and variation in predation rates among clones has been documented in several orchards (Askew et al 1985 a & b). Seed and cone insects can be major factors in determining the genetic composition of a seed crop and can alter the pollen contribution percentages as well as the seed contribution percentages. If a uniform pollen pattern is acquired but seeds are eliminated from the crop in a non-random or non-uniform pattern, the pollen contribution distribution is also effectively skewed. In fact, in orchards where the pollen contributions vary within the seed crops of individual clones, the alteration of gamete contribution proportions due to

insects will vary greatly between the pollen parent and the seed parent. Pest management practices may be costly and monitoring programs are often necessary to keep abreast of the damage and the effectiveness of the treatment.

D. PREVAILING WINDS

Orchards that are established in areas with prevailing winds at the time of pollen dissemination have a severe handicap. Downwind trees will certainly contribute less pollen to the seed crop than upwind clones. Buffer strips that are used as fall-out areas for pollen produced by neighboring trees need to be much wider on the upwind side of the orchard and may be of minimal value on the downwind side. Relative clone positioning throughout the orchard will need to be based on pollen dissemination distributions that are not uniform around the individual trees but are skewed toward the downwind side.

Pollen distribution patterns that are skewed by prevailing winds will severely affect the accuracy of the genetic evaluation techniques. Positioning of individual trees in a systematic design must be based on prevailing wind factors if pollen dissemination patterns are to be maintained at a desired level. Freeman (1967) suggested the use of balanced incomplete block designs to address this problem. Other designs such as random placement may be less susceptible to large deviations due to wind than a systematic design but will still be impacted by heavier foreign pollen doses on the upwind side of the orchard. It is imperative that poor pollen producers be shifted upwind relative to the heavy producers. Likewise, high quality clones should be positioned so the majority of their pollen falls within the orchard.

Monitoring studies are warranted for any orchard impacted by prevailing winds in order to quantify the degree of pollen drift and variation in wind direction and velocity during the pollination season.

E. POLLEN QUALITY

If variation in pollen viability or germination rate exists among the orchard clones, getting the pollen to the ovule will not assure the desired fertilization proportion. Instead, poor quality pollen sources will need to have greater pollen grain representation than high quality sources in order to boost the probability of attaining the desired fertilization percentages.

All orchards need to be tested on a regular basis to identify any severe variation in pollen viability. Orchard management practices can be altered or supplemental mass pollination may be implemented to alleviate low pollen potential in desired clones.

F. ADVANCED GENERATIONS

Advanced generation orchards may contain all of the problems of the first generation orchards plus an added problem. Almost certainly, the second or third generation selections will have higher coancestry levels than the first generation orchard. Relatedness among ramets that was the only source of inbreeding in the first generation orchard will be joined by relatedness among the clones. Full-sib and half-sib relationships could easily exist as could cousin and perhaps double cousin relationships. If inbreeding is to be avoided or minimized, additional placement constraints will be necessary. These constraints may exacerbate the problems of accommodating variation in phenology, pollen quality, insect predation and impacts of prevailing winds.

Selecting parent trees for advanced generation orchards solely on the basis of metric values to the exclusion of breeding characteristics may affect the realized genetic potential of the orchard. Monitoring studies of potential parents prior to selection would be useful to establish heritability of breeding traits and selection of phenologically conpatible trees will facilitate efficient cross-pollination and genetic balance.

G. SUPPLEMENTAL MASS POLLINATION

Pollen supplementation methods such as SMP can be effective only when the orchard manager has a complete understanding of his orchard phenology and other pollen management problems. Timing of SMP treatments may need to be tailored to individual clones or perhaps individual trees in higher generation orchards. The amount of genetic improvement to be attained by SMP is a function of the degree of replacement of undesirable pollen with desirable pollen and the differential of the ambient pollen clouds value and the SMP value. The problems mentioned earlier can cause great variation in the day-to-day value of the ambient pollen cloud and hence the degree of genetic improvement that is possible will vary from day-to-day. If severe variation exists in mating proportions, SMP might be needed to assure a high quality seed crop and may actually dominate the pollination percentages. If SMP is used in such an intensive manner as to effectively replace wind pollination within the orchard, then the SMP pollen composition needs to assure genetic diversity as well as genetic improvement. A program that utilizes only a few pollen sources for productions of millions of seeds would be questionable unless the genetics of the system were well known and GxE problems were not anticipated.

ESTIMATION OF GENETIC VALUES

All of the environmental and physiological limitations to pollen management can be accounted for in a mathematical estimation procedure. If quantitative data is lacking for a particular factor, the coefficients can be set at a conservative value until the data is gathered. Any number of estimation procedures are possible, but a simplified, straightforward approach is recommended here.

The average genetic value of seeds collected from clone j (S_j) in an orchard containing n clones is calculated as:

$$S_j = \frac{1}{2}\left(\left(\sum_{i=1}^{n} P_{ij} \cdot GV_i\right) + GV_j\right) \qquad (1)$$

where P_{ij} is the proportion of seed produced by clone i pollinating clone j and GV is the genetic value of clone i or j. P_{ij} is calculated as:

$$P_{ij} = \frac{PM_{ij}}{\sum_{i=1}^{n} PM_{ij}} \qquad (2)$$

where PM_{ij} is the relative probability of clone i pollinating clone j given flowering phenologies, pollen production levels, and pollen viability (after Askew 1988).

The genetic value of the orchard crop (GVO) realized by nursery production of seedlings from orchard seed is:

$$GVO = \sum_{j=1}^{n} \frac{S_j \cdot I_j \cdot V_j \cdot SP_j}{\sum_{j=1}^{n} I_j \cdot V_j \cdot SP_j} \qquad (3)$$

where I_j is the insect predation survival rate of clone j's seed crop; V_j is the germination percentage of clone j's seed crop; and SP_j is clone j's female strobilus production value.

In effect,

$$\frac{I_j \cdot V_j \cdot SP_j}{\sum_{j=1}^{n} I_j \cdot V_j \cdot SP_j} \qquad (4)$$

is the proportion of the orchards seedlings produced from seed of clone j.

It is interesting to note that of all of the factors that can influence the final genetic composition of the seed crop, only S_j is generally measured in most seed orchards.

Harvest proportions, after insect predation, can be calculated as:

$$I_j \cdot SP_j$$

Each factor should be specified at the outset of any orchard analysis and removed from the equations if it is found to be unimportant. Disregarding all of the potential limiting factors during the development of a pollen management strategy or in the process of roguing or design may lead to erroneous estimations of genetic value.

PM_{ij} is developed by studying strobilus production rates and flowering phenology for each clone. It may be based on several years of observation or recalculated for each season. Flowering induction techniques, supplemental mass pollination, orchard design and roguing will affect the value of PM_{ij}.

I_j may be estimated by insect damage surveys during cone harvesting. Insect control measures will affect the value of I and yearly fluctuations among individual clones should be expected.

V_j (seed viability) is usually not a problem in most orchard systems. However, in some instances sufficient variation may exist among individual seed lots to warrant its inclusion in genetic estimates. V's may be affected by seed handling techniques, orchard practices or vary due to inherent genetic variation and therefore it is a factor that may influence pollen management decisions.

SP_j is an important factor to monitor for orchard planning and genetic estimation. Final seed counts may be affected by a number of environmental factors that are either controllable or totally uncontrollable and unpredictable. In either case, it is important to have an understanding of the seed production potential of each clone in the absence of the modifying factors.

Overall, every variable is subject to modification either by natural fluctuations in the environment or by directed changes brought about by orchard management practices.

One final factor that affects the relative importance of the factors that influence the genetic value of the seed crop and the pollen management strategies is orchard size. Orchards with large numbers of clones (> 50) will generally be more stable in the presence of phenological or physiological variation among the clones. In effect, each clone in a 50 clone orchard is expected to contribute only 2% of the gametes and only very large variations in gamete contributions affecting many clones will cause an appreciable change in the overall genetic structure of the seed crop. Small orchards (<20 clones) are more susceptible to wide swings in genetic constitution. Significant changes in the gamete contributions of only 2 or 3 clones can greatly upset the distribution. Size acts as a buffer to clonal variation with respect to expected genetic values. The principle is similar to the problems of genetic drift and other small sample problems.

SUMMARY AND CONCLUSIONS

Pollen management strategies need to accommodate variation among clones in cone and seed insect susceptibility, gamete production, flowering phenology, seed and pollen viability and other factors that affect the final genetic composition of the seed crop. Orchard design, prevailing winds and other

environmental factors must also be considered during the planning of any management tactic or during the estimation of genetic value.

Accurate estimation of the final genetic composition of a seed crop will allow for effective planning for pollen management or supplementation. Orchard designs and parental selections can be used to minimize undesirable genetic problems and help the manager produce a consistent, predictable genetic composition.

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POLLEN DEVELOPMENT CLASSIFICATION SYSTEM FOR LOBLOLLY PINE

D. L. Bramlett and F. E. Bridgwater[1/]

Abstract.--A pollen development classification system (PDCS) for loblolly pine is presented that is parallel to the six-stage female development system currently used in tree breeding of southern pines. Ninety-four individual ramets at two sites in the Weyerhaeuser Company's loblolly pine seed orchard at Lyons, Georgia, were observed at 2-day intervals, and catkin development was scored from 3.0 to 6.0 during the period of March 7-30, 1988. In PDCS, Stage 3 covers development of individual sporophylls, Stage 4 is the start of pollen release, Stage 5 is maximum pollen release, and Stage 6 is the end of pollen release. Stages 3 and 5 are further subdivided.

With the system, duration of pollen release and percentage of pollen shed can be calculated for individual ramets in the orchard. Averages of the pollen development classes are then used to separate clones into early, middle and late pollen release in loblolly pine clones.

Keywords: Pinus taeda L., seed orchard, pollen distribution, forest genetics.

INTRODUCTION

As improvement of southern pines progresses with continuing cycles of breeding, progeny testing, selection, and establishment of advanced generation orchards, the genetic and economic value of the seeds increases. One of the basic assumptions of the program is that panmixia of the orchard breeding population occurs (Zobel and Talbert 1984). However, it is known that contamination from background pollen dilutes genetic gain from seed orchards and variability in clonal phenology prevents panmixia.

In addition, variability in numbers of male and female strobili lead to unequal parentage in the seed mix from a given orchard. In research studies and pollen distribution models, it is important to define the phenological development of pollen parents and to quantify the gametic production of each male ramet (Askew 1988). This paper reports a pollen classification procedure that relates catkin development to parallel stages of female flower development in loblolly pine (Pinus taeda L.).

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METHODS

Two areas of loblolly pine were selected in the Weyerhaeuser seed orchard at Lyons, Georgia. At each site, pollen catkin development was observed on all trees within a 90 foot radius of three target female parents. Pollen catkin phenology was classified and the proportions of pollen grains shed by each ramet were estimated for the 1988 pollen season. Fifty-seven ramets were observed at site 1 and 37 ramets observed at site 2.

Ten branch tips were tagged on each ramet, and a strip of flagging was attached to the branch for easy identification. Each branch tip included 1-6 clusters of pollen catkins (microspoangiate strobili). Three branch tips were located in the upper crown, four in the middle crown, and three in the lower crown. The branches were typically located in the aspect of the crown that was most accessible from the hydraulic lift truck. Since the sampled aspect varied from tree to tree, all aspects of the study trees were sampled. One to eight ramets of each clone appeared in the sample as a function of their frequency in the orchard at the sites selected for the study.

Catkin clusters were scored with a 6-stage pollen development classification system (PDCS) at the time of tagging and then at 1-2 day intervals for the remainder of the pollen season:

Stage 1.0 Catkins can be distinguished in the fall as individual buds on the vegetative shoot but are enclosed by bud scales.

Stage 2.0 Individual catkins emerge from their protective bud scales, and slowly elongate from November to February.

Stage 3.0 Individual microsporophylls develop as catkins increase in length over an extended period. Pollen catkins exude a clear fluid when pressed between the fingers.

- Substage 3.3 Pollen catkins exude a yellow fluid when pressed between the fingers. This stage occurs approximately 7-10 days before pollen release.
- 3.6 Catkins increase further in length and exude a clear fluid when pressed between the fingers. This stage occurs approxmately 3 to 5 days before pollen release.
- 3.9 Very little if any fluid can be pressed from the catkin. Microsporophylls are separating. Catkins bend easily and spaces are visible between sporophylls. Catkins feel light and "rubbery" when bent. This stage occurs approximately 1 to 2 days before pollen release.

Stage 4.0 Microsporophylls begin to release pollen. Pollen release begins at the proximal end of the catkin and progresses acropetally. Pollen released from cluster is less than 10 percent of the total.

Stage 5.0 Maximum pollen release for the catkin cluster. The majority of individual catkins within a cluster are releasing pollen. Pollen release can be divided into substages based on a visual estimate of the percent of pollen released from the catkin cluster.

- Substage 5.2 Twenty percent of the pollen released from the catkin cluster.
- 5.4 Forty percent released from catkin cluster.
- 5.6 Sixty percent of pollen released from catkin cluster.
- 5.8 Eighty percent of pollen released from catkin cluster.

Stage 6.0 Pollen release completed. All microsporophylls have opened and released pollen. Catkins are light to dark brown in color and are dry.

The 94 trees were scored with the PDCS from March 7 to March 30, 1988. When a cluster reached Stage 6.0, the individual catkins were counted and removed from the vegetative shoot. In all 27,072 individual catkins were observed on 2,108 clusters. The phenology of female flowers was also scored on the six target female sample trees using the standard 6-stage development procedure (Bramlett and O'Gwynn, 1980). The phenological scores were averaged for each ramet and clone and plotted over dates of observations.

RESULTS AND DISCUSSION

As the spring season progressed, the observation of pollen development on individual ramets increased from Stage 3.0 through Stage 6.0. The pattern of this development cycle followed a sigmoid curve with clones showing different starting dates (Stage 4.0), midpoint release dates (Stage 5.5) and completion dates (Stage 6.0). Figure 1 illustrates the range in development among clones.

These distinct pollen development patterns can have a large effect on the availability of pollen to the female flowers of specific clones in the orchard. For cross pollination to occur among specific seed orchard clones, it is obvious that the receptive stage of the female parent must coincide with pollen release of the male parent.

The primary advantage of the pollen development classification system is that it permits an estimation of proportions of pollen released over time rather than simply a record of whether pollen is being shed or not.

For example, female strobili on clone 081069 progressed through Stages 4.5-5.6, during which most pollinations are expected to occur (Bramlett and O'Gwynn 1980), from March 22 through March 25 (Figure 2). Males 071053 and 071059 had released essentially all pollen before March 22, thus would not have pollinated female strobili on clone 081069. The other three males contributed to the pollen pool in different proportions during the period of female receptivity. Males 061025 and 081077 released 80% and 40%, respectively, during the 4 days of interest; while male 081005 released no more than 10% during the last 2 days of the same period. Thus, the PDCS scores permit a refinement of estimates of the proportionate parentage among males that qualitative scores would not.

Synchronization of male and female development on the same tree was compared on the six designated female target trees (Table 1). From these observations it appears that designated pollen development class when described as a number, may be either ahead or behind female flower development class on the same ramet depending on the individual clone. For example, in clone 71022, female flower development class was consistently ahead of the pollen class, whereas in clone 81069, the female development class was consistently behind the pollen class number.

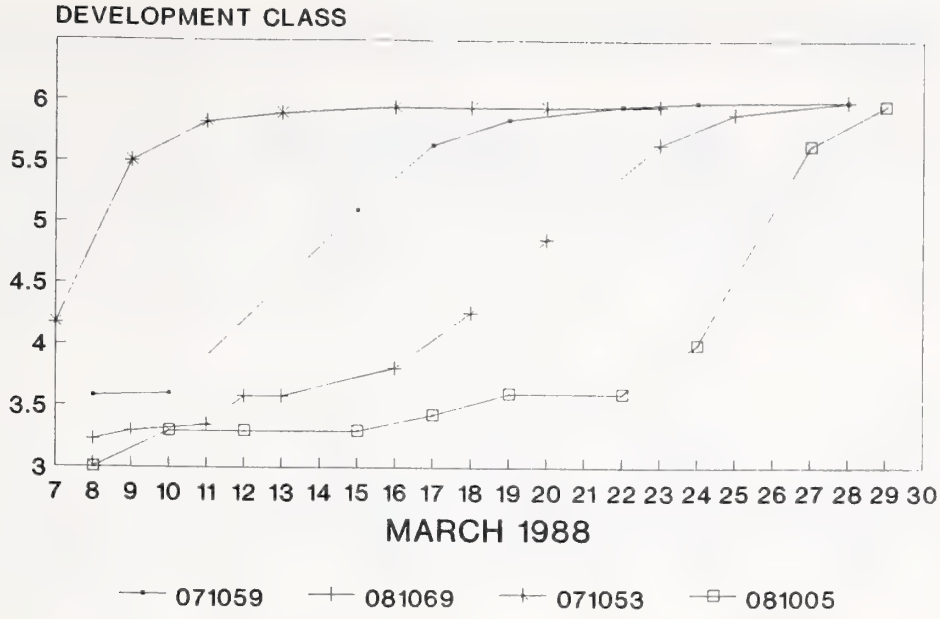

Figure 1. Average pollen development stage during March 1988 for four loblolly pine clones in the Loblolly Pine Seed Orchard, Weyerhaeuser Company, Lyons, Georgia.

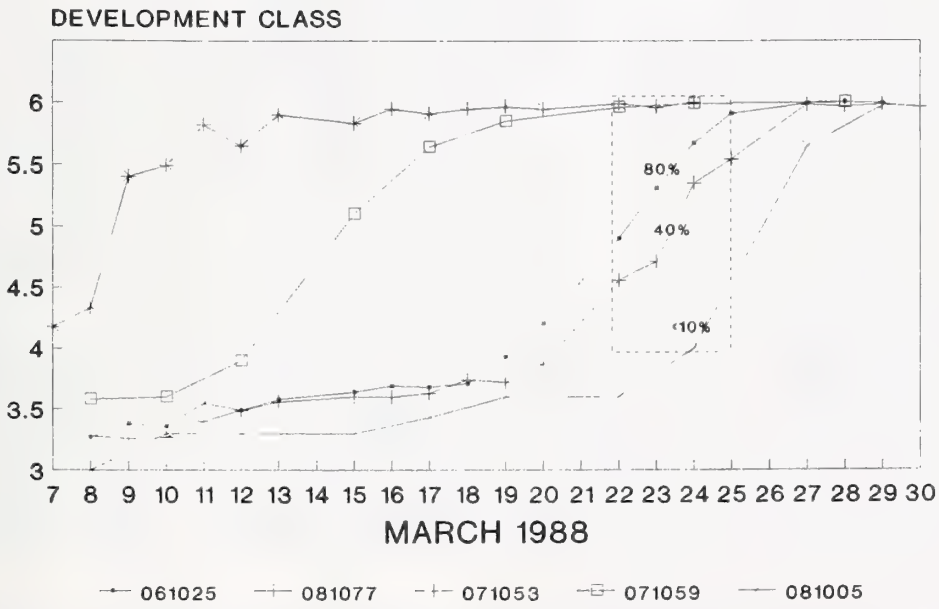

Figure 2.--Female strobilus receptivity for clone 081069 compared to catkin development and pollen release of five neighboring males. The period of maximum receptivity for the female clone (March 22-25, 1988) is enclosed in the rectangle.

Table 1.--Average scores of male and female development stages for six loblolly pine clones in the Weyerhaeuser Seed Orchard, Lyons, Georgia.

CLONE	MALE	FEMALE	DEVELOPMENT STAGE MALE	FEMALE	MALE	FEMALE
021021	4.0	4.5	5.0	5.3	5.5	5.9
061031	4.0	3.7	5.0	4.5	5.5	4.8
071022	4.0	4.6	5.0	5.6	5.5	5.8
081069	4.0	3.6	5.0	4.3	5.5	4.6
081086	4.0	3.3	5.0	3.9	5.5	4.2
AVERAGE	4.0	3.9	5.0	4.7	5.5	5.1

These differences may be partially explained by the fact that the classes themselves are artificial values that are assigned to the class for identification only. Nonetheless, assigning a number to the class does allow us to average the value for a specific date and consequently to make comparisons between clones. One problem with the PDCS is that in actual practice Stage 5.0 the onset of rapid pollen release, cannot be exactly identified. Pollen release begins from an individual cluster in Stage 4.0. Designation of that stage implies that only a very small fraction of the pollen contained in the entire cluster is being released. The next identifiable class would then be 5.2, when 20 percent of the pollen has been released from the entire cluster. It is conceivable to use 5.1 or 5.05 to indicate smaller amounts of pollen release, but in practice it would be difficult to give an estimate of the amount of pollen dispersed in percentages less than 20 percent.

In observing pollen release, the normal pattern is that the more proximal catkins on the cluster begin pollen release and progressively release pollen over time acropetally. Individual catkins also begin pollen release at the proximal part of the catkin and progress acropetally. On an individual tree, a rather wide diversity of pollen development stages were observed. For example, once a tree began shedding pollen (at least one cluster classified as 4.0), shedding continued for an average of 10 days until all clusters and reached stage 6.0.

Once individual clusters reach Stage 4.0, they moved rapidly through Stages 5.2 - 5.6. The time required for a ramet to move from an average of Stage 4.0 to 5.0 was about 3 days. Another 2 days were required for the average to reach 5.5 and then in 2 more days the average was 5.8. To reach a time when all clusters were 6.0 was somewhat longer because of the delay of

some individual catkins. For practical purposes, however, when the average reached 5.8-5.9, pollen dispersal was essentially completed for a tree.

These numeric classes are not quantitative variables. Nevertheless, when they are averaged they appear to fairly accurately reflect loblolly pine pollen dispersal. Variation among ramets of the same clone was remarkably low. Even from different sites in the orchard, pollen release values agreed very closely for ramets of the same clone. Thus, the conditions that influence pollen catkin development must have been fairly constant within the orchard, and the PDCS is repeatable.

CONCLUSIONS

The PDCS appears to reflect the development pattern of loblolly pine pollen dispersal, and to be highly repeatable. Clones can be ranked on the basis of their pollen release dates. When key pollen development stages are averaged for a ramet, Stage 4.0 equals the beginning the of pollen release, Stage 5.5 equals mid-point of pollen release, and Stage 6.0 equals cessation of pollen release.

Using PDCS, pollen release dates can be compared to female receptivity periods and proportionate paternity can be predicted for ramets or for an orchard as a whole.

Since PDCS scores were consistent among ramets in the seed orchard, it may be possible to use a small number of trees to reflect annual variation in pollen dispersal for a clone. However, the minimum numbers of observations to describe the pollen dispersal pattens for ramets, clones, and orchards have not been determined. If the dispersal pattern can be related to weather variables, extensive observation of catkins may not be needed to predict the pollen release patterns for ramets, clones, or a seed orchard.

ACKNOWLEDGEMENT

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CHARACTERIZING REPRODUCTIVE PHENOLOGY OF SEED ORCHARD CLONES

T. D. Blush[1/]

Abstract.--Phenological observations were made on fifteen clones in two first-generation loblolly pine seed orchards. A Coastal seed orchard was observed in 1986; a Virginia Piedmont seed orchard was observed in 1988. A method for illustrating clonal patterns of female and male reproductive phenology is presented. Within-clone variation in reproductive phenology was generally low. Within-tree variation was greater among crown levels (upper, middle, lower) than it was by crown aspect (north, south). These data are discussed from a seed orchard management perspective.

Keywords: Pinus taeda L., seed orchard, flowering, phenology.

INTRODUCTION

As Southern seed orchards mature, seed production has increased so that seed orchard managers can now be more concerned with the genetic quality of the seed orchard crop. A seed orchard with clones of high breeding values has the potential to produce high quality wind-pollinated seed. This potential is realized when conditions essential for achieving genetic efficiency are met (El-Kassaby et al. 1984): (a) minimal outside pollen contamination, (b) equal female/male strobili production, (c) equal cross-compatibility, (d) flowering synchronization, (e) random mating, and (f) minimal self-fertilization. Flowering synchronization is a condition of major consequence. Female/male reproductive phenology is an important determinant of random mating and can influence the degree of self-fertilization (Griffin 1984, El-Kassaby et al. 1988). Characterizing reproductive phenology of clones in established seed orchards can provide the manager with information useful for a variety of management activities. Breeding activities can be efficiently scheduled. Clonal patterns of reproductive phenology can be useful additional information when making thinning, roguing, and cone collecting decisions. Bridgwater et al. (1987) have found supplemental mass pollination to be more effective when done on clones receptive before peak seed orchard pollen flight. It may also be possible to modify genetic gain estimates based on a clone's mating relationships with its cohorts. Information on phenological patterns of seed orchard clones is thus of increasing value as we attempt to improve the genetic quality of seed orchard seed.

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MATERIALS AND METHODS

Study Material and Sampling Strategy

In 1986, fifteen clones comprising a first-generation Coastal loblolly pine (Pinus taeda L.) seed orchard were observed. In 1988, fifteen clones of managerial interest in a 24-clone first-generation Virginia Piedmont loblolly pine seed orchard were observed. Two ramets of each clone were selected from interior orchard positions. Selection was done to facilitate repeated observations from a mobile aerial lift. Both seed orchards are located near Charleston, South Carolina.

On each study tree, 20 female- and 30 male-bearing observation branches were selected and tagged for repeated observations. With two study trees, a total of 40 female and 60 male observation branches was selected per clone. One-half of the observation branches on a tree were located on the south side of the crown, one-half on the north side. On each crown side (north and south), observation branches were distributed among the upper, middle, and lower crown levels in proportion to the amount of strobili located in that region of the crown. For the fifteen clones observed in 1986 and 1988, a total of 1500 observation branches was selected. Of this total, 600 were female and 900 were male. More observation branches were allocated to males than to females because male strobili clusters appear to be more variable in their phenological patterns than females.

Phenological Observations

Observations were made in a systematic order on all observation branches during a day's census. A census was taken at two- to four-day intervals until all clones ceased to be receptive or shed pollen. Receptivity of female observation branches was scored using the 1 to 6 rating system of Bramlett and O'Gwynn (1980). Strobili clusters on male-bearing observation branches were scored 1 if shedding pollen or 0 if not shedding pollen when the branch was lightly tapped. Due to the somewhat subjective nature of determining female receptivity, one person conducted all censuses during an observation year. From the phenological observations of female and male observation branches of a clone, the proportions of receptive female observation branches (stages 4L to 5L) and of shedding male observation branches were calculated for each census date. These proportions were then used to construct "phenograms" for each clone in which the duration of female receptivity and pollen shed are represented by bands on a time line. The width of the band represents the proportion of strobili receptive or shedding pollen on a particular census date (Figure 1).

RESULTS AND DISCUSSION

Patterns of Reproductive Phenology

Figure 1 illustrates the periods and intensity of female receptivity and pollen shed respectively for fifteen first-generation Virginia Piedmont seed orchard clones observed in 1988. The upper half of each phenogram represents the proportion of receptive females or shedding males observed on ramet 1, the

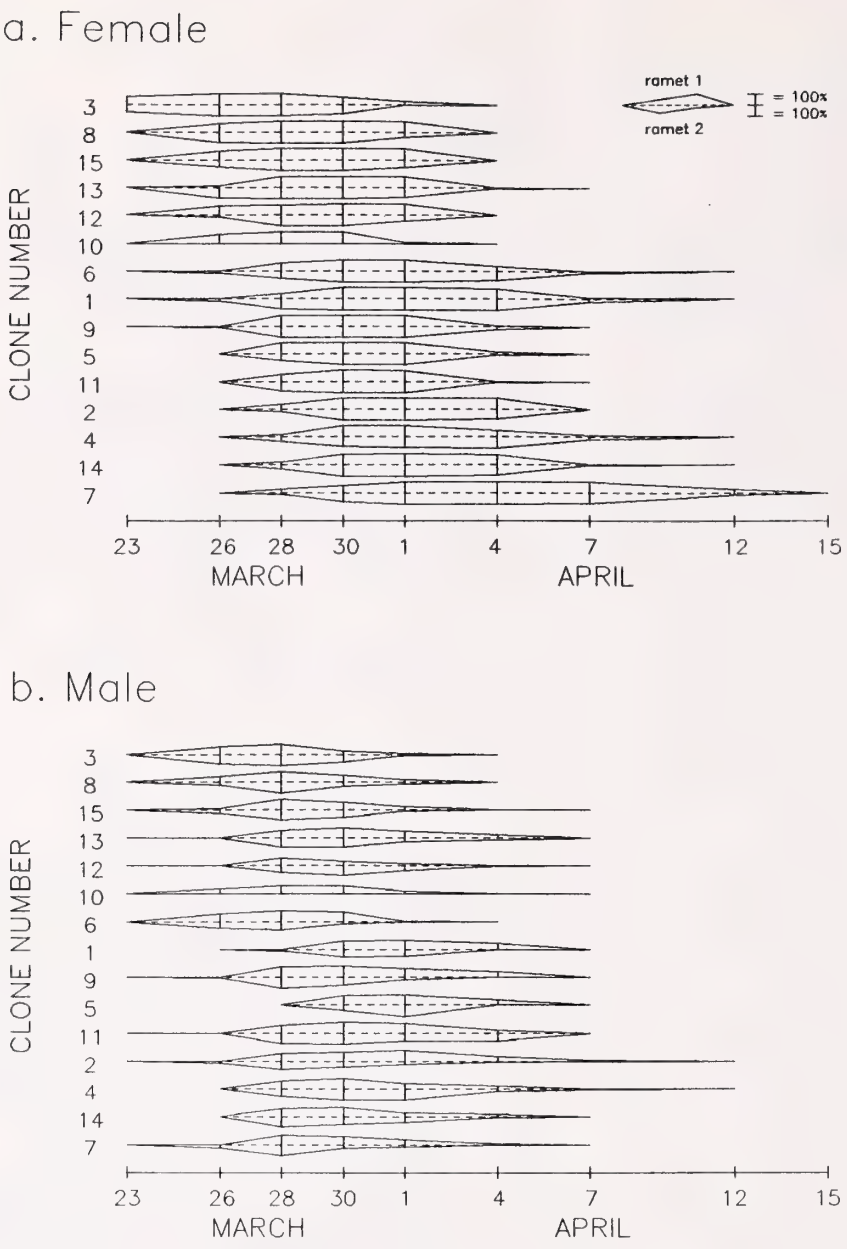

Figure 1. Female (a) and male (b) reproductive phenology of fifteen Virginia Piedmont loblolly pine seed orchard clones in 1988. The width of a horizontal band represents the percentage of strobili receptive on the indicated census day for each of two ramets. Observation branches were located on north and south crown aspects and stratified amongst lower, middle, and upper crown levels. Clones arranged in order of their female receptivity period, earliest to latest. Ramet two of clone 10 was eliminated from the study due to ambiguous clonal identification.

lower half represents ramet 2. These observations have proven quite useful for seed orchard management activities. It is important to verify that clones managed as an intermating reproductive population are, indeed, well synchronized with one another. This is especially important when seed orchard clones are relocated to regions far from the ortets' native range as is the case for some of the clones depicted in Figures 1 and 2. Relocation may result in reproductive anomalies (Schmidtling 1987). Our study clones were generally well synchronized. Clone 3 is classified as an "early" clone, while female receptivity of clone 7 is "late" and of extended duration. The remainder of the clones are classified as "middle". We have found these data useful for scheduling breeding activities, devising pollen management strategies, and as an adjunct to thinning and roguing decisions.

Figure 2 plots the percent of all female observation branches receptive and all male observation branches shedding pollen during 1986 in a first-generation Coastal loblolly pine seed orchard. On March 26, female receptivity peaked with 80% of the observation branches receptive and pollen shed peaked with 65% of observation branches shedding pollen. During peak reproductive activity we have documented a greater proportion of receptive female observation branches than there were shedding male observation branches. This pattern reflects the tendency in loblolly pine for individual female strobili clusters to remain receptive over a longer time span than that over which pollen shed occurs in male strobili clusters.

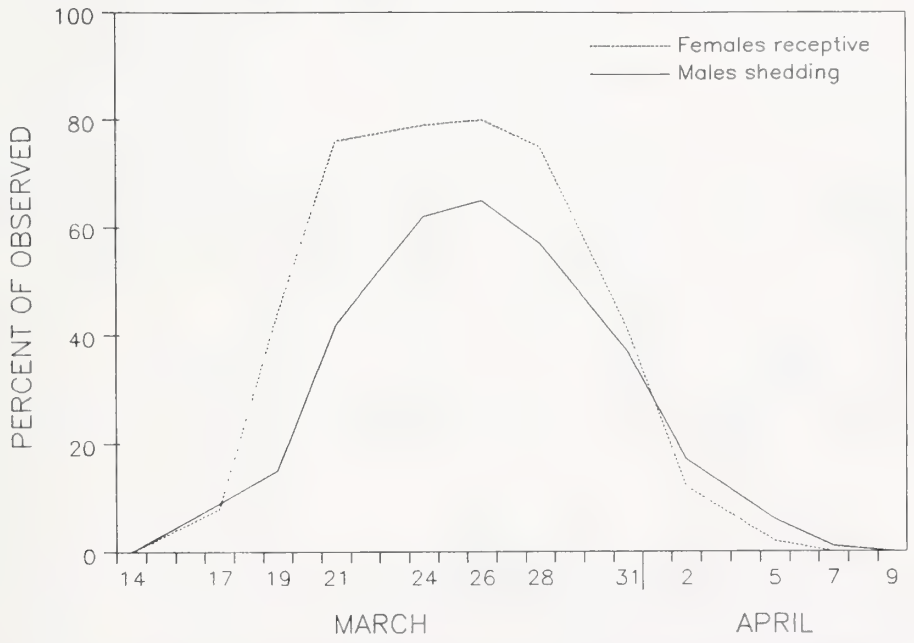

Figure 2. Percent of all female observation branches receptive and all male observation branches shedding pollen. Observations made on indicated census date during the 1986 reproductive period in a fifteen clone first-generation Coastal loblolly pine seed orchard.

Influence of Crown Aspect and Crown Level on Reproductive Phenology

Differences in phenological patterns between north and south crown aspects were usually not pronounced. Figure 3 illustrates this for 1988 observations of female strobili on Virginia Piedmont seed orchard clones. Male strobili in 1988 tended to be more variable, with a trend for north-side strobili to shed pollen over a longer time span than south-side strobili. In 1986 this trend was not apparent, however.

Differences in phenological patterns by crown level were more pronounced and consistent. Figure 4 presents observations taken in 1988 of female strobili (Figure 4a) and 1986 observations of male strobili (Figure 4b). The proportions of receptive female strobili located in the upper and middle crown levels were higher than those in the lower crown level at early census dates. A similar pattern was observed in 1986 observations. Male observation branches showed no differences at the beginning of pollen shed, but there was a trend for males in the upper crown to complete pollen shed before those at mid-crown level, and mid-crown males, in turn, shed pollen before lower crown males (Figure 4b). Similar trends were also seen in Virginia Piedmont clones in 1988. These trends in pollen shed are a reflection of the number and size of male strobili clusters by crown level. Male strobili clusters in the lower crown tended to be larger than those in the upper crown, and thus shed pollen for a longer time period due to their larger size (i.e. more and larger strobili per cluster).

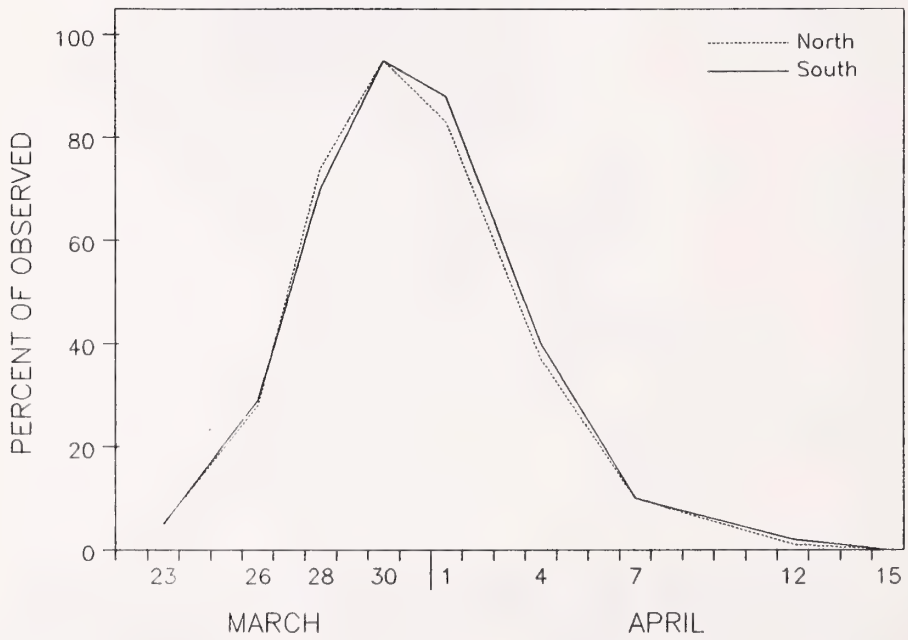

Figure 3. Comparison of female receptivity between crown aspects. Observations made on indicated census date during the 1988 reproductive period on fifteen clones in a first-generation Virginia Piedmont loblolly pine seed orchard. Percent of all receptive female observation branches on north and south crown aspects is shown.

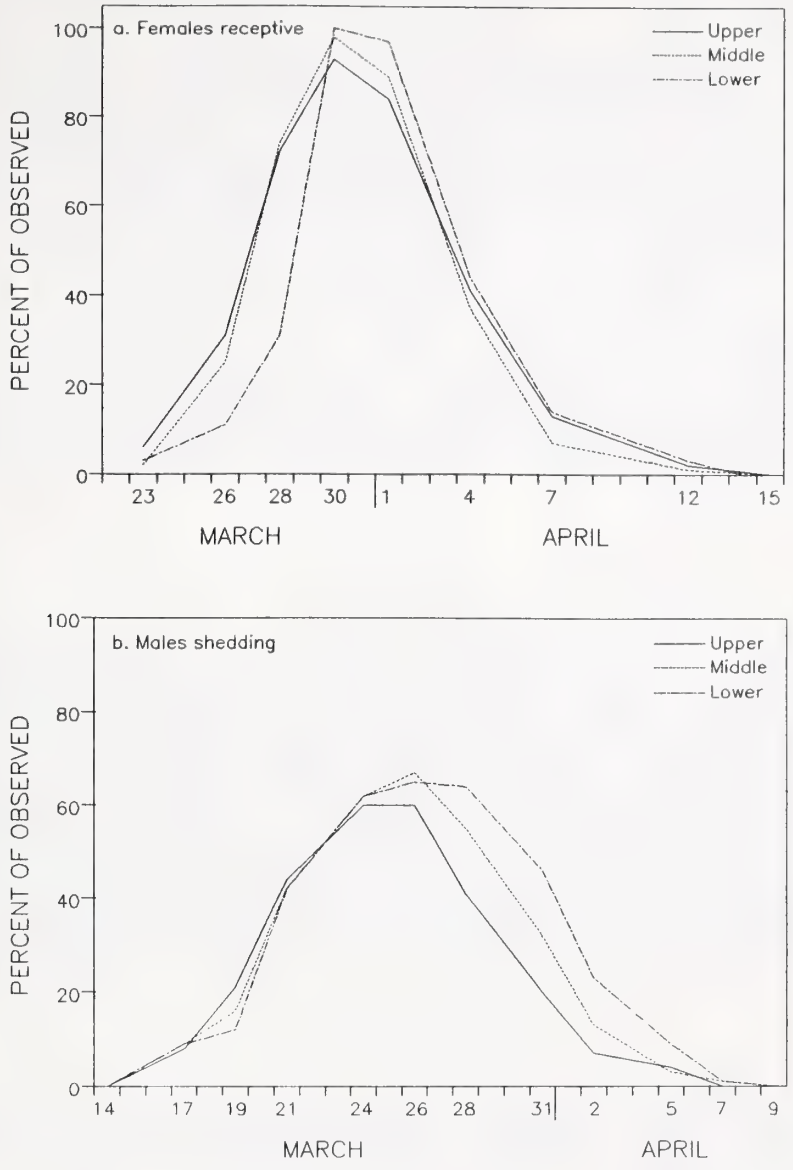

Figure 4. Comparison of female receptivity and male pollen shed between crown levels. (a) Percent of all receptive female observation branches in upper, middle, and lower crown levels on indicated census date during the 1988 reproductive period in a first-generation Virginia Piedmont loblolly pine seed orchard. (b) Percent of all shedding male observation branches in upper, middle, and lower crown levels on indicated census date during the 1986 reproductive period in a first-generation Coastal loblolly pine seed orchard.

These observations suggest that within-crown patterns of female reproductive phenology may be determined more by branch hierarchy than by the microclimate experienced in different crown regions. I know of no literature on within-crown influences on reproductive phenology. In the absence of experimental data, some speculations can be made based on patterns of physiological function within tree crowns. The position of a reproductive branch in the crown determines its nutritional and water stress status, and these factors probably influence the timing and pattern of receptivity. Female strobili on rapidly elongating upper-crown branches become receptive sooner than those on subordinate lower-crown branches (Figure 4a). Rapidly elongating upper-crown branches are a strong nutrient sink and have favorable water relations. This should benefit accompanying female strobili by shortening their time to ripen under permissive environmental conditions. Due to their variability and the confounding of cluster size with crown level, it is difficult to interpret within-crown patterns of pollen shed in male strobili from the observations of these studies. The influence that branch hierarchy and related physiological differences have on patterns of reproductive phenology is not well understood. This would be an interesting area in which to do further research.

CONCLUSIONS

The results of these and other studies have shown the value of characterizing patterns of reproductive phenology in conifer seed orchards. Phenograms are a convenient way to characterize the intensity and duration of female receptivity and pollen shed for a collection of clones, and they facilitate comparison among clones. Clones not synchronized with the rest of the seed orchard can be readily identified. For the seed orchard as a whole, female strobili clusters appear to remain receptive over a longer time span than male strobili clusters. Within-crown variation in female reproductive phenology appears to be greatest by crown level, with little differences by crown aspect. The timing of pollen shed within the crown is highly variable. More intensive sampling is needed to determine within-crown patterns (if any) of pollen shed.

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EFFECTS OF CROWN PRUNING ON HEIGHT AND CONE PRODUCTION BY LOBLOLLY PINE AFTER 6 YEARS

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and
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Abstract.--The effects of several different types of crown pruning, begun at different tree heights, on flowering by grafted loblolly pine seed orchard trees were evaluated periodically for a 6 years. Some of the pruning methods were based on those commonly used on fruit trees, where bowl-shaped crowns result from removal of vertically growing shoots. Unfortunately, even pruning twice a year for several years did not often result in a fruit tree type crown because loblolly pine has extremely strong apical control. Even when horizontal growth by several vigorous lateral branches was encouraged, one of them usually became vertical and suppressed the growth of the more horizontal branches. The overall result of our treatments was a significant decrease in height growth and a correspondingly greater decrease in cone production. Even on those few trees that developed bowl-shaped crowns, the width of the crown made harvest of interior cones difficult. We conclude that the benefits of height control, even if begun early, are more than offset by decreases in cone production.

Key Words: Loblolly pine, seed orchards, crown management, cone harvest, seed orchard management.

OBJECTIVE

The objective of this study was to determine whether the height and shape of the crowns of trees in a loblolly pine (Pinus taeda L.) seed orchard could be managed by pruning, to reduce cone harvest costs. Lift trucks with 60-foot booms have been required for most of this decade to harvest cones in Weyerhaeuser's North Carolina first generation seed orchard which was established in 1959. Our hypothesis was that we could duplicate the results of pruning in fruit tree orchards, where tree height is limited by forcing the crown into a bowl-shaped habit, so that cone harvest would be possible with smaller and less expensive man lifts. Crown management has been attempted for a number of conifers with mixed success (see literature review by Gerwig 1987). Studies on Southern pines have shown that pruning reduces cone production (e.g., van Buijtenen and Brown 1962; Varnell 1969), and led to a recommendation from the

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North Carolina State Tree Improvement Cooperative that crown training be avoided (Jett 1978). However, pruning was not repeated in several successive years, nor was it begun early in the life of the tree. Our rationale was that pruning, begun early, would significantly limit tree height without affecting cone production. Gerwig (1987) has reported on a similar study, also carried out on loblolly pine, which was begun in 1982. The study reported on here was begun in 1980, and our results will be compared with those of Gerwig (198').

METHODS

The study area is located in Weyerhaeuser's Lyons, NC loblolly pine seed orchard near Vidalia, Georgia, where 10 clones were established in rows during 1977 and 1978, at 15-x 30-foot spacing. The area was divided into 6 blocks across clonal rows so that each block represented 3 rows of 10 clones each. Three treatments were randomly assigned to each of these rows, which included (1) an unpruned control, (2) pruning begun when the trees reached a height of 2 meters (m) or more, and (3) pruning begun when the trees reached a height of 3m or more. A total of 180 trees were treated.

Pruning was begun in February, 1980, starting with removal of the entire terminal long shoot, down to the first branch whorl from the previous year's growth. Since almost no trees were >3m in 1980, only treatment 2 was applied; treatment 3 began in 1981. More elaborate pruning treatments, which included disbuding of vigorous lateral branches as well as terminal shoot removal, had been evaluated in a previous study (Bramlett and Greenwood 1982, unpublished data, Weyerhaeuser Co., Hot Springs, AR 71901), but did not significantly augment the effects of leader removal on crown shape or tree height. Consequently, disbuding was abandoned after 1981. In the years following the first pruning, dominant terminal shoots were removed from any lateral branches that had become vertical. In practically all the trees, the pruned terminal shoot was rapidly replaced by 1 or more lateral shoots from the first whorl of branches, which became vertical in the growing season after pruning and effectively replaced the terminal shoot. Consequently, pruning in 1981, 1982 and 1983 involved removing those shoots, which were often as large as those removed at the first pruning. Since lateral shoots became dominant so quickly, pruning removed as much as 1/3 of the total crown in the first 2 years of the study, and drastically (but temporarily) altered the appearance of the trees. Height and diameter were measured immediately after pruning in 1982 and 1983, and female flower buds were counted, if present, on any branches removed. Total counts of female flowers were made in late March, before needle elongation occurred. After pruning in February, 1983, the trees were allowed to recover for several years, and the effects of the treatments on height, diameter and flowering were evaluated in March, 1986. All results were analyzed using analysis of variance. The model used included replication, clone and treatment as main effects, and all two-way interactions. Three-way interactions were not possible since within each replication, each clone-treatment combination was represented by only 1 tree.

RESULTS AND DISCUSSION

Prior to pruning in 1980, ANOVA showed no significant difference in tree heights or diameters among the trees selected for the 3 treatments. In 1982,

1983 and 1986, the effects of clone and treatment on height and female flowering were highly significant ($p < .008$ for both clone and treatment). In 1986 only, replication did have a significant effect on tree height ($p < .01$) but not on female production ($p < .20$). The interactions between clone, treatment and replication for height and flowering were not consistently significant across years but significance was occasionally observed (see Table 1). Therefore, we can conclude that pruning significantly decreased height growth and flowering in 1982, 1983 and 1986 (see Table 2). Although the expected differences in flowering among clones were observed, the effect of pruning on flowering was the same on poor and good flowering clones (see Table 3), which is reflected in the absence of significant clone x treatment interactions ($p < .63$) in 1986.

Table 1. ANOVA table for height growth and female cone production in 1983 and 1986.

Effects	dF	Height (1983) MS	Height (1983) F-ratio	Height (1986) MS	Height (1986) F-ratio	Cones (1983) MS	Cones (1983) F-ratio	Cones (1986) MS	Cones (1986) F-ratio
Rep	5	22787	1.07	54385	3.23	26655	1.47	44569	1.55
Clone	9	146266	3.80	444237	14.68	844587	25.83	1700528	32.78
Trt	2	581373	67.97	315833	46.95	64817	8.92	59536	5.16
Rep*Clone	44	245680	1.31	173337	1.17	154719	0.97	454146	1.79
Rep*Trt	10	41826	0.98	33598	1.00	24017	0.66	29170	0.45
Clone*Trt	18	90847	1.18	60573	1.00	137742	2.11	89016	0.86

Table 2. Overall effects of pruning on height, diameter and cone production by grafted loblolly pine seed orchard trees in 1982, 1983 and 1986.

		Treatment 1 (control)	Treatment 2 (prune >2m)	Treatment 3 (prune >3m)	% Reduction from control 2 vs. 1	% Reduction from control 3 vs. 1
Ht+(cm)	'82	392	254	262	35%	33%
	'83	545	390	412	28%	24%
	'86	775	665	680	13%	12%
Dia+(mm)	'82	100	88	94	12%	6%
	'83	142	116	119	18%	16%
	'86	219	198	197	10%	10%
Cones(#)	'82*	41	20	42	51%	0%
	'83*	107	51	50	52%	53%
	'86	145	94	98	39%	36%
# Times Pruned		0	4	2-3	--	--

+Measured immediately after pruning.
*Includes # cones on branches pruned off.

Table 3. Clonal response to pruning treatments for height and number of female flowers in 1986, 3 years after pruning was discontinued. Different letters indicated means differ at $p \leq .05$.

	Height (cm)			# Female Flowers		
	1	2	3	1	2	3
Clone	Control	Prune >2m	Prune >3m	Control	Prune >2m	Prune >3m
1	710	656	663	183	95	119
2	775	720	753	113	30	44
3	756	668	653	20	0	5
4	712	579	588	400	398	377
5	740	633	634	72	107	54
6	821	721	678	259	162	191
7	780	491	606	32	22	5
8	856	766	768	141	6	37
9	773	682	688	27	14	26
10	823	728	724	202	108	119
$\bar{X}$	775a	664b	676b	145a	94b	98b

Thus, overall, pruning decreased height growth by 12 to 13%, and female flowering by 36 to 39% in 1986 (see Table 1), even though the trees had 3 growing seasons to recover from the final pruning. Unfortunately, the residual inhibitory effects of pruning were about 3 times greater on flowering than on height growth, which was just the opposite of the desired effect. Gerwig (1987) also reports no residual inhibition of height growth, noting that pruned trees put on just as much height growth as the unpruned control in the growing season following pruning. He concludes that pruning only limits height growth in the year that it is applied, and that flowering is reduced in proportion to the amount height is decreased. Our results support this conclusion, but show that pruning has a more lasting inhibitory effect on flowering than on height growth. Since the trees in Gerwig's study were still being pruned, the differential effect reported here on height and flowering several years after pruning stopped awaits confirmation.

In addition to a slight reduction in height, we were successful in forcing the horizontal development of 1 or more large lateral branches between 1 and 3m off the ground. However, the terminal shoots of these branches are now growing upwards, and observations in December, 1987, indicate that it is more difficult to work around the pruned trees than the controls. The large, low lateral branches make approach to the main stem difficult, and the crowns of the pruned trees appear to cover more area than the controls. Thus, more movement around the crown would be required for cone harvest, and reaching the interior of the pruned crowns is more difficult. Also, the large lateral branches may be more susceptible to ice damage.

RECOMMENDATIONS

Based on the results reported here, we do not recommend pruning of loblolly pine seed orchard trees during early development. Loblolly pine has a very strong tendency to restore apical control following removal of terminal shoots, so the effects of even repeated pruning on height growth are temporary. This, plus the apparently greater inhibitory effect on flowering, do not justify the effort and cost needed to control height. We would, therefore, only recommend that top pruning be applied when the trees outgrow the vertical capacity of available manlifts. When that occurs, removal of cone-bearing branches just above reach during cone harvest is advised. A single top pruning (pollarding) of relatively large radiata pine seed orchard trees resulted in increases in flowering several years after pruning. Young trees, however, did not show increases in flower production from a single pruning (Pederick and Brown, 1976).

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SEED ORCHARD TURF MANAGEMENT WITH SUBLETHAL APPLICATION OF SELECTED HERBICIDES

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Abstract.--In March of 1987 and 1988, sublethal applications of Roundup, and Oust in various combinations were applied to the turf of five different orchards. In general, grass and seed growth were significantly reduced compared to untreated control plots. Oust (0.021bs/acre) and an Oust-Roundup mix (Oust 0.02/lbs/acre, Roundup 3.0 oz./acre) proved to be the most effective in retarding grass growth. In each study year, chemical control of grass growth diminished in late July and August. The results of this study suggest that it may be possible to eliminate orchard mowing until July, thus, the equipment operator is available to complete other job assignments.

EVALUATING POLYPROPYLENE BULK BAGS AS PINE CONE COLLECTION CONTAINERS AND COMPARING THEM TO WIRE BOUND WOODEN CRATES

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Abstract.--Slash pine (Pinus elliottii Engelm.) and loblolly pine (P. taeda L.) pine cones were harvested from seed orchards during September and October of 1988. All cones were placed in either 20 bushel wire bound wooden crates or 20 bushel polypropylene bulk bags and shipped to International Forest Seed Company for seed extraction and conditioning. Seed yields (pounds per bushel) and seed quality as expressed by speed of germination (PV), total percent germination and the percentage of fungus infection were evaluated for each container type. The costs and benefits of using each container in the seed orchard and seed plant are discussed.

Keywords: Pinus elliottii Engelm., Pinus taeda L., polypropylene bulk bags, wooden crates.

INTRODUCTION

Seed orchard managers are always looking for greater efficiency in their seed production. One of the most costly operations is cone harvesting. Those who continue to hand pick must confront the issue of the type of containers they use to hold cones before they are processed. It is logical to choose the container that provides a safe haven against seed degradation resulting in poor seed yield and seed quality. But, because they are managers they too must consider all the costs involved ranging from purchase price and depreciation to handling costs in the orchard and processing plant.

In the southeast the "one bushel" burlap bag and the twenty bushel wire bound wooden crates have been successfully used as containers for all the southern pines. Barnett, 1979b compared burlap bags and crates for storage of slash pine but neither container showed a consistent advantage. A study by F. T. Bonner, 1987 showed that neither container type had a significant affect on loblolly germination nor were they significantly different in yielding full clean seed.

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At International Forest Seed Company, we have been investigating the use of polypropylene bulk bags as a container to hold, transport, and store cones before processing.

Flexible intermediate bulk containers or "bulk bags" originated in Europe and Asia where they were developed over 20 years ago to hold agriculture seeds such as dried grain. They are now used extensively in the grain and flour industry in the U. S. as well as for bulk handling of industrial chemicals, fertilizers and cement.

Our interest in these bags as cone containers was enticed by the immediate recognition of their durability and ease of handling (at least when they were empty). The first use by IFSCO was a "square" bag (23 cubic feet) about the same size as a 20 bushel crate. Our initial comparison between a bulk bag and a crate revealed acceptable germination data (91% vs. 90%) using loblolly cones. We therefore proceeded to modify the bag for equipment handling and have since developed two models: A bottom discharge bag with straps attached to the top for lifting purposes and a solid bottom bag with side sleeves to enable the bag to be lifted and rotated 180^{o} to empty. Each has their advantages and disadvantages and are discussed in a later section of this paper.

In a 1987 operational study at IFSCO of Slash pine, 760 bushels of cones in bulk bags were compared against 850 bushels in crates for percent germination and seed yield. The results were extremely promising showing 1.47 pounds per bushel and an average 96% germination for cones stored in bags and 1.43 pounds per bushel and an average and germination of 94% for those held in crates. A loblolly pine operational study of 1,000 bushels collected in 1987 resulted in a yield of 1.39 pounds per bushel and an average germination of 96%.

Based upon these operational results we felt confident that seed quality would not suffer if cones were stored in polypropylene bags and that they could be substituted for crates or burlap bags. However, we wanted to document the bulk bag's usefulness so we installed a replicated study comparing the uses of crates and bulk bags and the effects on seed yield and quality.

METHODS

Loblolly and slash pine bulk cone collections were made from grafted seed orchards during the weeks of October 10 and September 13, 1988, respectively. All cones were assumed to be mature (.88 specific gravity or less for loblolly, and .90 for slash) since each species were harvested well into their respective seasons. Enough cones were harvested from the loblolly orchard to fill 10 crates and 10 bags each with 20 bushels. Cones from the slash orchard were more scarce and only 185 bushels were included in the study, enough to fill 5 bags with 17 bushels each, and 5 crates with 20 bushels. For each species, all crates and bags were

uniquely identified so that individual containers were treated as separate lots through processing and seed testing. The tops of the bulk bags remained untied (except when in transit to our processing plant and excessive periods of rain) and allowed to "season" as do cones in crates. Barnett, 1979b showed that germination was faster in loblolly seeds from cones stored in the open: for slash there was no difference and in shortleaf (P. echinata mill.) increased both germination rate and total germination. All the bags were set on pallets to provide air circulation underneath each bag. Slash cones were placed in the kiln for 60 hours at 105°F, after 10 weeks from harvest. Two hundred bushels of loblolly cones (100 from crates and 100 from bags) were kiln dried at 105°F for 48 hours 10 weeks and 15 weeks from harvest. The drying, seed extraction and cleaning were done on an operational basis in an attempt to mimic as best as possible operational conditions. Seed yields for each container were recorded to the nearest hundredth of a pound.

Seed testing was completed in our laboratory according to official testing standards (Association of Official Seed Analysts, 1981). Twenty-eight day un-stratified germination tests were performed on all slash pine lots while for loblolly 28 day 35°F wet stratification germination tests were performed. Radiographs of each lot were examined for fungi infection. To compare the seed vigor of each lot the peak value was calculated according to Czabator, 1962 and Bonner, 1986. The percent germination data and the percent of fungus infection data was transformed using the arcsin transformation so ANOVA could be applied. A completely randomized design was used and an ANOVA applied to all four variables. The length of cone seasoning and container treatments were treated as fixed effects.

RESULTS

Slash pine seed yields were not significantly different ($P < 0.5$) for cones stored in crates (1.07 pounds per bushel) or bulk bags (0.99 pounds per bushel). However, differences in the seed quality evaluations for slash pine were significant (Table 1). Germination percent was an average 12% lower and the percent fungus infection was 8% higher from seed extracted from cones stored in bulk bags (Table 2). The Peak Value (PV) (the germination rate divided by the number of days at that count) was also lower for seed originating from bulk bags (PV = 5.04) and significantly different from seed originating from crates (PV = 6.3).

Table 1. Mean squares and F-values for seed yield, percent germination, percent fungus infection and germination rate (PV) for slash pine.

Source of Variation = Container Type[1]			
Variable	Df	MS	F
Yield	1	0.010	2.86 NS[2]
% Germination	1	436.890	19.80 *[3]
% Fungus infection	1	237.170	25.76 *
Germination Rate	1	4.10	19.60 *

[1]Container types are fixed effects.
[2]NS indicates no significant differences P > 0.05.
[3]* indicates significant differences P > 0.05.

Table 2. Average seeds per pound, percent germination, percent fungus infection, and peak value for slash pine and loblolly stored in crates or bulk bags.

	Seeds/Lb.		% Germination		% Fungus Infection		Peak Value	
	Crate	Bulk Bag	Crate	Bulk Bag	Crate	Bulk Bag	Crate	Bulk Bag
Slash	1.07	0.99	95	83	3	11	6	5
[1]Loblolly (10)	1.22	1.18	98	94	1	2	7	6
[1]Loblolly (15)	1.39	1.35	98	97	2	3	11	10

[1]Loblolly (10) and Loblolly (15) were held in crates or bulk bags for 10 or 15 weeks after harvest and before extraction.

For loblolly seed the results are a bit more promising in regards to the effects of the bulk bag on cone storage. Since we had twice as many bushels of loblolly than slash pine, we were able to process half the cones (100 bushels in 5 crates and 100 bushels in 5 bulk bags) after 10 weeks of seasoning and the same amount after 15 weeks (Table 3). Seed yields were not significantly different between container types within storage time. But, yields from the containers after 15 weeks of seasoning (1.39 pounds per bushel for crates and 1.35 pounds per bushel for bags) were significantly greater than those after 10 weeks (1.22 pounds per bushel for crates and 1.18 for bulk bags). Differences among the three seed quality characteristics measured are present in some situations. The average percent germination is high in both cases (Table 2) but was significantly lower from seed extracted from cones stored in bulk bags (94% after 10 weeks and 97% after 15 weeks) than those in crates (98% after 10 and 15 weeks). Also, germination was significantly greater after 15 weeks of storage (98% for crates and 96% for bulk bags). The average percent fungus infection was significantly greater in seed originating from cones stored in bulk bags (2.5% vs. 1.5%) but not significantly different within or between storage times. Like the percent germination the Peak Value was slightly greater from seed associated with crates but was not significant. PV was significant however after 15 weeks of storage time.

DISCUSSION

Slash Pine

The apparent damaging influence the bulk bag had on the slash seeds was surprising. This is the first occurrence in which we have recorded lower values as compared to crates. The difference in seed quality was probably due to the difference in weather from year to year. The rainfall was noticeably higher in the fall months of 1988 (12.58 in.) than during 1987 (7.41 in.). The mean temperature of 53.5^{o}F and 53.0^{o}F were about normal. Wet weather when coupled with warm temperature is undesirable when harvesting and storing any crop.

We may expect to have a lower level of air circulation in the bulk bag because of the obvious construction and material differences but the polypropylene weave is breathable and patterned after a weave used to store grain. The fabric is a 9 ounce agritainer used by the agricultural industry. Standing water never occurs in the bag but the cones may remain wet longer than those in crates.

The factors that probably have the greatest influence in regard to the germability of pine seed are those related to dormancy. Slash pine does not enter a very "deep" dormancy state. Those biological/chemical factors which keep the seed active also influence the susceptibility of the gametophyte tissue to resist infection as well as the vigor to germinate. The most prevalent

diseases presently known to infect slash pine seed are Diplodia gossypina and Fusarium moniliforme var. subglutinans (T. Miller and D. L. Bramlet, 1978). It is obvious from these data fungus infection influence not only total germination but also the germination rate (PV).

These data point to the long standing belief that cone handling has a strong influence on seed quality (Bonner, 1987; Bonner, 1984, Barrett, 1976, 1979b; McLemore, 1975, and others). Harvesting, handling, and storage must be customized in regards to the weather particulars of each season no matter what storage system the orchard manager chooses. Bulk bag storage of slash cones requires different handling procedures than when crates are used. At this point the information available is inconclusive and we will continue testing the bulk bags. Those who continue to use bulk bags must at least set them on pallets, and tie the tops when it rains and open the tops when the sun is shining.

Loblolly

It appears from this study that length of storage seems to affect loblolly seed quality more so than the containers. Although yield differences were not significant for either effect the trend of higher germination after 15 weeks of storage is quite evident. The percent germination and germination rate (PV) were significantly better after 15 weeks of storage as compared to 10 weeks. This trend was found by F. T. Bonner, 1987 when loblolly cones were stored outdoors in crates or burlap bags. Apparently additional maturation is occurring in these seeds during cone storage. Bonner, 1987 described the effect as creating a physiological equivalent of seed storage. The percent fungus infection was not significantly different. This is important to know because during heavy crop years it is not uncommon for cones to sit in storage well into February or March.

The fungus infection was significantly greater in seeds extracted from cones stored in bags. I suppose one might expect the increase of fungus growth over time especially during warm and wet conditions.

The lack of significant interactions is encouraging in that both containers perform consistently in regards to the variables studied in this experiment.

Cost Comparisons - Crates vs. Bulk Bags

Wire bound wooden crates have been a labor saver during harvest time for all of those who use them. It's the yearly maintenance and storage of these crates that has been the nemesis. Based upon the experiences we have encountered during the past 4 years, it is in this area of seed orchard management that using bulk bags as cone collection containers can make immediate improvements.

Bulk bags are easy to handle. They last a long time and require a fraction of the storage space of crates as well as the yearly maintenance (Table 4). For those who freight cones to our processing facility, full bags cost the same as full crates. But the returning empty bags requires a fraction of the cost that is necessary to return crates. We have loaded 150 empty bags (3,000 bushel capacity) into the back of a pick-up truck with room left over. The managers that are presently using bulk bags have had to modify only slightly their normal cone collection procedures to accommodate their differences. The most obvious difference between wooden crates and the bulk bags is their lack of rigidity. The bags should be held open until after the first 4 or 5 bushels provides enough "bulk" to hold them open on their own.

To move and load the sleeve type bulk bags a bobcat loader is probably necessary. They have the maneuverability to "spear" both sides at once. Tractor mounted forks work fine to move the bulk bags with loops attached to the top. It is possible to obtain a bag with both lifting devices. The sleeves lend the bags to 180° rotation when emptying the cones during processing.

The handling of the full bags in the orchard and the seed plant requires a bit more time to stage, lift and load (or empty). Like crates, two bags at a time can be loaded on to a flatbed truck for transporting but it requires a little extra time due to the care taken to "spear" the sleeves or loops. During extraction the average time to retrieve and empty a crate of cones tumble then, clean the system and prepare for another lot was 19 minutes, the same for a bulk bag with sleeves. But the bag with the bottom discharge requires more time and is not particularly liked by the processing crew; cleaning, folding and storage of the sleeve type bag is also much easier.

Table 3. Mean squares and F-value for seed yield, percent germination, percent fungus infection and germination rate (PV) for loblolly pine.[1]

Source of variation	Df	MS	F
SEED YIELD			
Storage time	1	.133	3.89 NS[2]
Container type	1	.008	0.25 NS
ST x CT	1	.000	0.00 NS
Error	16	.034	0.00 NS
GERMINATION			
Storage time	1	24.865	5.08 *[3]
Container type	1	71.821	14.67 *
ST x CT	1	4.901	1.00 NS
Error	16	4.895	
PERCENT FUNGUS INFECTION			
Storage time	1	4.141	1.14 NS
Container type	1	17.485	4.83 *
ST x CT	1	0.013	0.00 NS
Error	16	3.620	0.00 NS
GERMINATION RATE (PV)			
Storage time	1	63.013	13.97 *
Container type	1	2.381	0.53 NS
ST x CT	1	0.013	0.00 NS
Error	16	4.512	

[1]Storage times and container types are fixed effects.
[2]NS indicates no significant differences at P > 0.05.
[3]* indicates significant differences at P > 0.05.

Table 4. Cost comparisons for using 20 bushel bulk bags and wire bound wooden crates as pine cone storage containers.

Item	Dollars	
	Bulk Bags	Crates
Annual Depreciation[1]	200	350
Storage and Repair[2]	141	814
Harvest Handling[3]	16	36
Freight[4]	261	422
Cost per Year/1,000 bushels	618	1,622

[1]Assume 50 bags @ $20.00 lasting five years and 50 crates @ $21.00 lasting three years.
[2]Assume $5.00 per square foot, bags occupying 25 square feet and crates 150 square feet; 4 man hours to maintain bags, 16 man hours to maintain crates @ $4.00 per hour.
[3]Assume 4 man hours of handling bags and 9 man hours to handle crates @ $4.00 per hour.
[4]Assume 380 mile distance empty bags occupying 25 square feet, crates 150, costs of $21.00 and $182.00; full bags or crates @ $240.00 per 1,000 bushels.

CONCLUSION

There are no significant differences between the pounds of seed extracted from loblolly or slash cones stored in crates or bulk bags. In each case the percent germination, percent fungus infection and germination rate was lower and in some cases significantly at the 5% level from seed that originated in bulk bags. This indicates as expected that cones in bags must be handled differently when in storage. Presently the bulk bag with sleeves and loops are preferred over the bottom discharge bag and costs are considerably lower for bulk bags as compared to crates. Future test are necessary to determine the proper seasoning methods when cones are stored in bags to ensure high yields as well as high quality seed.

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FACTORS AFFECTING THE INCIDENCE AND DEVELOPMENT OF BLACK SEED ROT OF SLASH PINE CAUSED BY LASIODIPLODIA THEOBROMAE

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Abstract.--A study was initiated in the fall of 1988 to determine the incidence of diseased seed occurring in seed orchard trees before cone harvest, and the impact of cone harvesting practices on the incidence of seedborne fungi and the development of disease in seed. Four ramets of four slash pine clones (16 trees total) were selected in a north Florida seed orchard. Cones were harvested from each tree on three dates (8/31, 9/14, 9/26) and were divided among three treatments:

1) No Ground Contact/No Storage (NGC/NS) - cones handpicked, placed individually in #5 kraft paper bags, and immediately dried at 38 C for 48 hrs;
2) No Ground Contact/Storage (NGC/S) - cones handpicked, placed individually in #5 kraft paper bags, stored for 5 wks in a ventilated building without temperature control, then dried at 38 C for 48 hrs;
3) Ground Contact/Storage (GC/S) - cones handpicked, dropped to the ground, picked up 72 hrs later and placed individually in #5 kraft paper bags, stored for 5 wks, then dried at 38 C for 48 hrs.

Seed extracted from each cone were radiographed and assessed for the percentage of seed that were filled, empty, fungus-damaged, and damaged due to other causes. A sample of seed from each cone was subsequently plated on two types of agar media to determine fungal associations with seed. A sample of cones was obtained from each tree at each sample period for determination of specific gravity.

Disease was absent, and seedborne fungi were essentially nonexistant in seed obtained from cones in the NGC/NS treatment. The incidence of cones with diseased seed in the NFC/S treatment was low for all harvest dates (range, 20 to 10 percent). In contrast, the percentage of cones with diseased seed was high (55%) in the GC/S treatment for the 8/31 harvest date. Cones of all clones were highly susceptible when harvested at this time (range, 42-75% of cones with diseased seed). At the 9/14 harvest date, the incidence of cones with diseased seed in the GC/S treatment declined for all clones (range 0-17%) with the exception of one clone (ID-007, 58%). On the 9/26 harvest date, only 4% of all cones contained any diseased seed in the GC/S treatment (range among clones, 0-8%). Seed from individual cones with a high incidence of fungal-type damage yielded almost exclusively Lasiodiplodia theobromae. The results of this study provide evidence that cone maturation (i.e. specific gravity) at harvest may be critical for cone colonization and seed infection by fungi which cause disease in slash pine seed.

CONCURRENT SESSION: 1A

A CHLOROPLAST DNA PROBE IDENTIFIES UNEXPECTEDLY HIGH LEVELS OF POLYMORPHISM IN PINUS BANKSIANA AND PINUS CONTORTA

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Abstract.--A cloned, 500 base pair (bp) restriction fragment from the chloroplast genome of Pinus contorta hybridized to restriction fragments of a previously known polymorphism when used in molecular mapping experiments with total cellular DNAs of P. banksiana and P. contorta. Unexpectedly however, a large number (as many as 20) of additional, extremely polymorphic, DNA fragments were also identified by this probe. Study of a large number of individuals of P. banksiana and P. contorta, using the cloned 500 bp P. contorta fragment, leads to the following conclusions: 1) in random samples from across the allopatric ranges of the two species, as well as in samples from two populations of a sympatric region shared by P. banksiana and P. contorta, many individuals have identifiably unique autoradiogram patterns; 2) in a restricted set of germplasm, representing seven individuals from a single P. banksiana provenance, nearly every individual has an identifiably unique genotype; and 3) at least some of the highly-variable bands appear to reside in the chloroplasts. The 500 bp probe may be useful for clonal identification in tree improvement programs. Because of the inheritance of chloroplasts through pollen in conifers, this cloned fragment may also be suitable for paternity resolution.

Keywords: jack pine, lodgepole pine, DNA fingerprinting, hypervariability, RFLP.

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INTRODUCTION

Genetic markers that could unambiguously distinguish all genotypes in populations of forest trees would be of significant value in forest genetics. If such markers became widely available in forest trees, as they are in animals (Jeffreys 1987), then clonal identification and paternity analyses (e.g., Adams et al. 1988) would no longer need to rely on presently available, often ambiguous, genetic markers such as allozymes. Unambiguous markers would also provide unprecedented precision for studies of evolutionary processes in natural populations.

A recent report in the literature suggests that it may be possible to extend the most powerful methods of genotypic identification (DNA fingerprinting) to plant species (Rogstad et al. 1988). These authors presented preliminary results for several taxa, including the forest trees Pinus torreyana, Populus deltoides and Populus tremuloides.

The data reported below support the notion that DNA fingerprinting may indeed be possible in the genus Pinus, using sequences from the chloroplast genome as probes. However, the identity of such probes may differ among taxa within the genus.

METHODS

Genetic Material

The material studied was obtained from both natural and experimental populations of Pinus banksiana and P. contorta in Canada, the United States and Sweden. Details regarding the origin of this material have been published previously, and the specific relevant citations are provided below in the figure legends.

Analyses of DNA Polymorphisms

Total cellular DNA was purified from each individual as described by Wagner et al. (1987), and chloroplast (cp) DNA was purified from individual trees by the method of Szmidt et al. (1986). Restriction fragments produced by double digestion (to completion) with the restriction endonucleases BamHI and SstI were separated by electrophoresis through agarose, transferred to Biotrans membranes, and visualized by Southern (1975) hybridization and autoradiography. The ^{32}P-labeled (Feinberg and Vogelstein 1983) probe for the molecular hybridizations was a 500 bp BamHI - SmaI fragment, cloned from the region between psbA1 and psbA2 in the chloroplast genome of P. contorta (Lidholm et al. 1988).

RESULTS AND DISCUSSION

High Levels of Detectable Genetic Variation

The chosen combination of restriction endonucleases and probe permitted the visualization of many bands in each DNA sample. These bands produced a large number of autoradiogram patterns in samples drawn from the allopatric and sympatric ranges of P. banksiana and P. contorta. Moreover, in a sample of seven individuals of P. banksiana from a single provenance, at least six different genotypes were evident (e.g., Figure 1).

Figure 1. Autoradiogram of total cellular DNA samples, doubly digested with BamHI and SstI, probed with the 500 bp cpDNA fragment cloned from P. contorta. The lanes contain (left-to-right): samples from seven different individuals of P. banksiana from a single provenance in Ontario, Canada (lanes 1 - 7); samples from three different individuals of P. contorta from a single provenance in British Columbia, Canada (lanes 8 - 10); and samples from five different individuals of P. banksiana - P. contorta from a single population in a sympatric region in Alberta, Canada (lanes 11 - 15). The fragment sizes range approximately from 500 bp to 4500 bp in size. The DNA samples represented on the autoradiogram originated from studies reported elsewhere (Wagner et al. 1987; Govindaraju et al. 1989; Wagner et al. 1989).

Subcellular Location of the Polymorphism

Two lines of evidence suggest that at least some of the highly-variable bands must reside in the chloroplasts of P. banksiana and P. contorta. First, these variable banding patterns of P. banksiana are inherited paternally in a controlled cross (data not shown), as is cpDNA in P. banksiana (Wagner et al. 1989) and other conifers (Neale and Sederoff 1988). Second, the 500 bp probe is homologous to many fragments in DNA purified from chloroplasts of P. contorta (Figure 2). This second observation indicates that a sequence within the probe fragment may occur several times in the chloroplasts of P. banksiana and P. contorta.

Figure 2. Autoradiogram of DNA purified from chloroplasts, doubly digested with BamHI and SstI, probed with the 500 bp cpDNA fragment cloned from P. contorta. The two samples were prepared from individuals of P. contorta, growing in Sweden and originating from two provenances in Yukon Territory, Canada. These individuals are two of the plus-tree selections described by Fries and Lindgren (1986).

Potential Pitfalls and Limitations

The diversity of autoradiogram patterns identified could be explained by several factors. For example, small recombination products of the chloroplast genome, resulting from homology among short repeats, might persist in the chloroplasts and be detected by autoradiography.

In principle, the polymorphism may also be due to differential, incomplete enzyme digests. However, repeated DNA purifications from three single trees produced consistent results among samples within trees, and all progeny from a controlled cross of *P. banksiana* consistently exhibited their paternal genotype. These two facts are evidence that the high level of polymorphism documented by Figure 1 is not the result of random partial digestions.

We have screened individuals of four other species in the genus *Pinus* (*P. ponderosa*, *P. resinosa*, *P. rigida*, *P. taeda*), using the same probe that reveals polymorphism in *P. banksiana* and *P. contorta*. Although this screen revealed *inter*-specific differences, polymorphism did not occur *within* any of the other four species studied. This indicates that probes which identify hypervariability are likely to differ among taxa in the genus *Pinus*.

CONCLUSIONS

Regardless of the precise molecular mechanism which generates the polymorphism we have discovered, its potential utility is obvious. A genetic marker, which is consistent within individuals and known pedigrees, yet which unambiguously distinguishes many individuals in populations of forest trees, offers great power for the solution of problems associated with clonal identification, paternity resolution, and population/evolutionary genetics. The hypervariable markers we have identified may be particularly significant because of their paternal inheritance through chloroplasts.

Clearly, more work is needed to determine the frequencies of the hypervariable genotypes in natural and breeding populations before these markers could be recommended for application. Additionally, we can do no more than speculate that similar markers exist in other plant species. Nonetheless, the present example, involving small repeated sequences in the chloroplasts of *P. banksiana* and *P. contorta*, may be instructive for systematic searches for hypervariable markers in other coniferous taxa.

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MOLECULAR PROBES FOR DROUGHT STRESS IN TWO GEOGRAPHICALLY-SEPARATED POPULATIONS OF LOBLOLLY PINE

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Abstract.--Many native-plant species which grow in a variety of environments have evolved mechanisms for survival under chronic and acute water deficits. Some of these mechanisms have cellular and metabolic components. Osmotic adjustment, changes in metabolic pathways, appearance of new proteins, and production of polyamines have been proposed as components by which plants adapt to drought stress. Synthesis of molecular probes from successful species will facilitate our understanding of the basic cellular components which contribute to the adaptations of these species.

Messenger RNA was prepared from polysomes or total RNA. Polysomes were isolated from needles of loblolly pine (*Pinus taeda* L.). From the polysomes, mRNA (polyA$^+$ RNA) was separated by digestion with proteinase K and chromatography over oligo d(T) columns. Total RNA was isolated by LiCl precipitation, and mRNA isolated from Hybond-mAP. Total and mRNA's were translated *in vitro*. PolyA$^+$ RNA was used to synthesize cDNA and to construct cDNA libraries in lambda ZAP, which were subsequently converted to plasmids. Several clones were used in Southern analyses of restricted genomic DNA. The cDNA libraries from control and stressed seedlings from two geographically-separated populations will be screened for clones which are homologous to clones for drought-stressed genes from pea. Unique clones in stressed libraries will be selected by subtractive hybridization.

Keywords: *Pinus taeda* L., molecular probes, cDNA, drought-stress

INTRODUCTION

Native species have evolved mechanisms to withstand abiotic stresses; temperature (Newton and Goodin, 1989b), chronic and acute periods of water deficits (Newton and Goodin, 1989a), metals (Tomsett and Thurman, 1988), anoxia (McLeod, et al., 1986), etc. (Sach and Ho, 1986). Herbaceous plants also have evolved similar mechanisms and responses to drought stress (Jones et al., 1981). Some of these mechanisms have cellular and metabolic components. Plants adapt to drought stress through response mechanisms such as osmotic adjustment (Newton et al., 1986a), reduction in DNA content and enhanced tissue elasticity (Castro-Jimenez et al., 1989), changes in metabolic pathways with the accumulation of compounds such as proline, (Bhaskaran et al., 1985; Newton et al., 1986b, 1987), appearance of new proteins, (Hulbert et al., 1988; Valluri et al., 1988), synthesis of ABA (Guerrero and Mullet, 1986), and changes in transcription and translation (Guerrero and Mullet, 1988).

Synthesis of molecular cDNA probes from non-stressed (control) and drought-stressed plants can be used identify clones which represent polyA$^+$ RNA which are rapidly induced by drought stress. This report describes the synthesis of cDNA libraries from loblolly pine and the strategy for their use.

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METHODS

Plant Material

Stratified seeds of loblolly pine,*Pinus taeda*, L. (Texas Superior, MSSO 174; 1984M; Texas Forest Service) were germinated in moist vermiculite/peat mixture. Seedlings were grown under greenhouse conditions in flats. Ten-gram aliquots of needles were harvested and frozen in liquid nitrogen (LN_2), ground in a mortar and pestle in liquid nitrogen, transferred to a coffee grinder with several pieces of dry-ice, ground further, and stored at -80° C until use.

Isolation of Polysomes

The ten-gram aliquots of ground tissue were extracted with grinding buffer (Wagner et al., 1987). After stirring with a spatula, the samples were squeezed through 8 layers of cheese cloth and centrifuged in 45-ml plastic tubes for 3 min at 27,000 rpm at 4° C. The supernatant fractions were transferred to a flask and 0.1 volume of 10% Triton X-100 were added. Aliquots were transferred to a Dounce homogenizer and homogenized (15 strokes). Pooled samples were centrifuged at 16,000 rpm for 7.5 min.

The supernatant fractions were layered over a step gradient (0.5 M sucrose with a 1.5 M sucrose in grinding buffer). After centrifugation at 27,000 rpm for 3 hours (SW 28 rotor), the supernatant fractions were aspirated, the walls of the tube washed and aspirated, and the cushion aspirated. The pellets which contained the polysomes were resuspended in 500 µl of polysome resuspension buffer and stored at -80° C.

Isolation of Total RNA

Nucleic acids from 10-gram aliquots of tissue were extracted as previously described (Wagner et al., 1987). Total RNA was precipitated with LiCl (Ausubel et al., 1987).

Isolation of PolyA$^+$ RNA

To 500-µl samples of polysomes, 500 µl of 1 M NaCl and 10 µl of 10 mg ml^{-1} proteinase K were added. After digestion at 25° C for 30 min, the samples were centrifuged for 2.5 min. The samples were heated to 65° C for 3 min and recentrifuged for 2 min and cooled to room temperature. The RNA was quantitated spectrophotometrically. Typical yield of RNA at this step was 2.5 mg. Oligo d(T) columns were prepared according to the manufacturers directions and samples loaded. The columns were washed with 0.5 M NaCl in TE (10 mM Tris, pH 8.5; 0.1 mM EDTA) until the A_{260} was less than 1.0 after which they were washed with 0.5 M KCl until the absorbance was less than 0.1. The polyA$^+$ RNA was eluted with four 400-µl aliquots of elution buffer. The RNA was ethanol precipitated, washed with ethanol, dried, resuspended in TE, and quantitated.

PolyA$^+$ RNA was also isolated from the total RNA fraction with Hybond-mAP® according to the manufacturers directions (Amersham).

Synthesis and Analysis of cDNA

Complementary DNA's (cDNA's) were constructed with several modifications of classical procedures. The first strand was synthesized with cloned MMLV reverse transcriptase. A

synthetic oligonucleotide was used instead of the classical oligo $d(T)^{12-18}$ primer, and it placed an *Xho* I site immediately downstream of the cDNA.

The second-strand synthesis was a modification (Gubler and Hoffman, 1983) of the RNAse H/DNA polymerase I/DNA ligase protocol (Okayama and Berg, 1982). The mRNA was removed by RNase H, and the second-strand synthesized with DNA polymerase I and DNA ligase. First- and second-strand synthesis were quantitated by determining the specific radioactivity of aliquots of each reaction. Reaction products were analyzed in alkaline-gels with ^{32}P end-labelled markers.

Preparation of cDNA Libraries

After synthesis of the second strand, the ragged ends of the cDNA were repaired with Klenow fragment of DNA polymerase. *Sac* I (isochizomer of *Sst* I) linkers were end-labelled with γ-^{32}P ATP. The labelled linkers were ligated to the cDNA with T4 ligase. The linkered DNA was digested with *Sst* I and *Xho* I and dephosphorylated. Unligated linkers were removed by chromatography. The linkered-cDNA was eluted into digested lambda ZAP DNA. After precipitation and resuspension of the linkered cDNA and ZAP arms, the two were ligated with T4 ligase, and the ligated DNA was packaged. This procedure insures that the cDNA inserts are cloned into the vector in the same orientation, a requirement which is necessary for subtractive hybridization.

RESULTS AND DISCUSSION

Total and polyA$^+$ RNA supported *in vitro* translation (Figure 1). As others have observed, translation products for the "total" RNA samples is of higher molecular weight than that of the polyA$^+$ RNA samples. This may reflect protection from RNase attack of mRNA in the "total" sample.

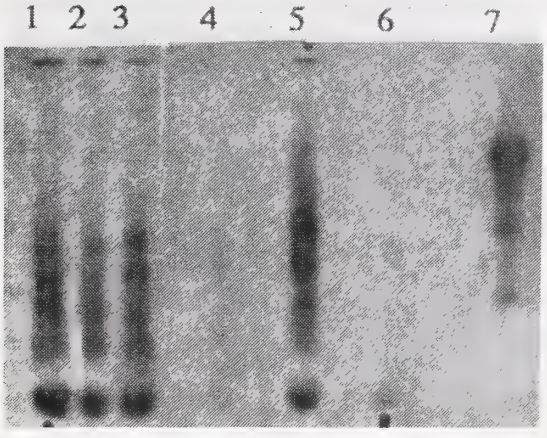

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Figure 1. *In vitro* translation products of total and polyA$^+$ RNA from loblolly pine. Total and polyA$^+$ RNA was translated in a rabbit reticulocyte system according to the manufacturer's instructions (Progema) with ^{35}S methionine as the label. Translation productions were separated by SDS-PAGE and visualized by autofluorography. PolyA$^+$ RNA, lanes 1-4; total RNA, lane 5; minus RNA control, lane 6; brome mosaic virus RNA control, lane 7.

PolyA$^+$ RNA from seedlings was used to construct cDNA libraries. Results of first and second strand synthesis of a typical experiment are shown in Figure 2. The bulk of the cDNA was between 500 and 2500 base-pairs in length.

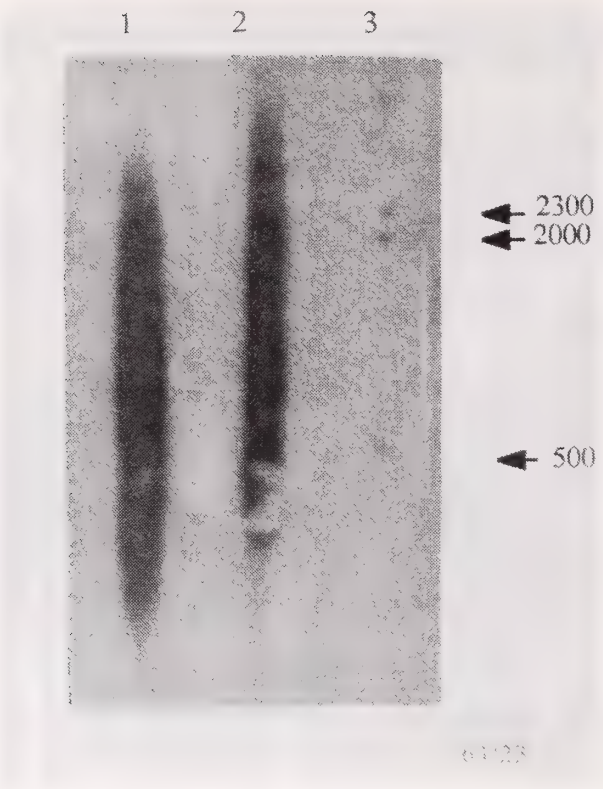

Figure 2. Synthesis of cDNA. Newly synthesized strands were end-labelled with ^{32}P and analyzed on alkaline agarose gel and visualized. Lane 1, first strand; Lane 2, second strand; Lane 3, standards. Arrows indicate the size of the standards in base pairs.

The directionally cloned cDNA was ligated to restricted lambda ZAP DNA and packaged. Table 1 summarizes the libraries constructed from seedling RNA. While the titer of these libraries is low, there is sufficient number of clones to begin screening. Several were selected as probes for Southern analyses.

Inserts from several clones from experiment 64/23 were labelled with ^{32}P by the random primer method according to the manufacturer's directions(Boehringer Mannheim). These probes were used in Southern blots of pine genomic DNA which was restricted with *Hind* III and *Eco R* I (Figure 3).

These data show the successful isolation of mRNA from loblolly pine and its use in the construction of cDNA libraries. Several clones from these libraries were used in southern analyses of restricted genomic DNA. This demonstrates their potential for RFLP mapping of loblolly pine.

Now that the method for routine construction of directionally cloned libraries has been established, libraries from mRNA isolated from stressed and control tissue will be constructed from seedlings of two geographically-separated populations of loblolly pine. By subtractive hybridization and differential screening, these libraries will be screened for clones which represent polyA$^+$ RNA's whose levels change in response to drought stress.

Table 1. Titer of cDNA libraries.

Experiment Number	total plaque forming units	percent transformants
64/17	7.7×10^4	2
64/23	1.1×10^3	99
64/32-59	0.3×10^3	64
64/32-64	0.2×10^3	87

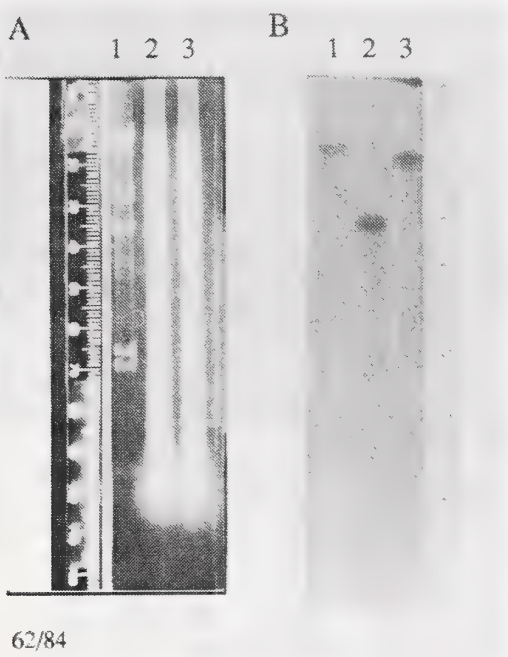

Figure 3. Southern blots of genomic DNA from loblolly pine. DNA was extracted from needles and restricted with *Hind* III (Lane 2) and *Eco R* I (Lane 3) and separated on an agarose gel along with standards (Lane 1). The DNA was transferred to nitrocellulose filters and probed with inserts from the library. A. Ethidium bromide stained gel. B. Nitrocellulose probed with clone from cDNA library.

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IN VITRO PROPAGATION OF PIN OAK

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Abstract.--Conditions were defined for in vitro propagation of pin oak (Quercus palustris Muenchh.). Competence of seedling explants averaged 93% on agar media compared to 41% in liquid culture; however, variable numbers (19-52%) of agar-produced shoots developed abnormally whereas normal shoot development occurred in liquid culture. Decreasing benzylaminopurine (BAP) from 8.9 to 0.089 µM in agar media did not affect competency but decreased the frequency of abnormal shoots and halved the yield of primary shoots. Shoots were subcultured in liquid modified Schenck and Hildebrandt or broadleaf tree media with 0.89 µM BAP. Eighty percent or more of the shoots could be rooted after indole-3-butyric acid treatment, but only plantlets rooted on filter paper bridges were successfully established in potting mix. Plantlets were acclimatized to greenhouse conditions.

Keywords: Quercus palustris Muenchh., micropropagation, tissue culture.

INTRODUCTION

Regeneration of trees and establishment of selected clones are necessary steps if various tissue culture strategies are to be effectively used in forest tree improvement programs. Notable success has been achieved with a few species in which trees have been regenerated from individual protoplasts, and clones of genetically transformed trees have ben developed (Haissig et al., 1987). However, for many important forest trees, such as oaks, suitable conditions for simple micropropagation from explants have not been defined.

Limited research on the improvement of oaks has revealed broad variation among individual trees in heritable growth traits (Cech, 1971: LaFarge and Lewis, 1987). The ability to clone select individuals would have obvious advantages, but traditional methods of grafting and rooting of cuttings have generally proved to be inefficient or unreliable for most species of oak.

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To overcome these problems, methods for tissue culture propagation have been applied with success to several Quercus spp. Several species have been micropropagated from seedling explants, and at least two species from explants taken from mature trees (Bennett and Davies, 1986; Meier-Dinkel, 1987; San-Jose et al., 1988).

Pin oak (Quercus palustris Muenchh.) is a major forest tree species on wet sites and heavy clay soils in the East and Midwest, particularly along the Ohio and Mississippi River drainages (Fowells, 1965). Although of limited timber value, it is a fast growing tree important for mast production in waterfowl habitat and is one of the most valuable ornamental oaks in the United States (McArdle and Santamour, 1987).

Lineberger (1980) reported callus development for pin oak but found no development of roots or shoots in culture. Bennett (1986) stated that pin oak could be micropropagated by methods developed for shumard oak (Bennett and Davies, 1986) but no details were given. In this paper we present results of research done to define parameters for in vitro propagation of pin oak and establishment of the derived plantlets.

MATERIALS AND METHODS

Seedling explants were taken from 4- to 12-week-old pin oak seedlings grown from stratified acorns sown in a commercial peatlite mix fertilized with Osmocote (17:7:12) and Micromax Plus (Sierra Chem. Co.). Seedlings were grown under a 16-hr photoperiod in a greenhouse. Stems were cleaned under running tap water, dipped in 95% ethanol for 30-90 sec and submerged in 1.0% NaOCl containing 0.04% Tween 20 for 20 min. After three rinses in sterile distilled water, single-node explants about 2.5 cm long were cut from the surface sterilized stems and placed in culture.

To obtain explants from mature trees, dormant branch terminals were cut during February-April and forced in a greenhouse under a 16-hr photoperiod. The branch terminals were kept in a solution of streptomycin sulfate (50 mg/l) and benomyl (10 mg/l a.i.) to discourage growth of bacteria and fungi. As new softwood shoots were produced they were harvested, surface sterilized as described above and cut into explants each with two or more nodes.

Explants were placed in 150 X 25-mm tubes containing either 14 or 3 ml of agar medium (0.6% w/v Difco Bacto agar) or 3 ml of liquid medium (Bennett and Davies, 1986). Broadleaf tree (BTM) (Chalupa, 1983), a modified Schenck and Hildebrandt (MSH) (Vieitez et al., 1985) and woody plant (Lloyd and McCown, 1980) media were used; all contained 3% w/v sucrose and were adjusted to pH 5.3. Media were supplemented with growth regulators as indicated prior to autoclaving. Explant cultures were placed under cool-white fluorescent lighting (40-70 $\mu E/sec/m^2$) with a 16-hr photoperiod at 26°C. Explants were transferred to fresh media after 2 days, and thereafter, 3 ml liquid and agar cultures were transferred weekly and 14 ml agar cultures every 3 wk.

Shoot subculturing and most research on rooting were done in GA-7 vessels (Magenta Corp.) containing 20 ml of liquid medium and four or five

explants or subcultured shoots. Temperature and lighting were as described above, and media were changed biweekly. In the rooting phase, three or four IBA-treated shoots were placed on filter paper bridges over 20 ml of medium in GA-7 vessels.

Secondary and tertiary shoots from subcultures were used in rooting experiments. Shoots were treated with indole-3-butyric acid (IBA) by (1) incorporating the IBA (3.7μM) into half-strength MSH with 3% sucrose and 0.6% agar,(2) dipping the shoots in 2.5 mM IBA for 15 min (Bennett and Davies, 1986) before culturing them on the same medium without IBA, or (3) a modification of the trea ment method of Zimmerman and Fordham (1985). In the latter method, three or four shoots were placed in tubes with 1.0 ml of half-strength MSH or BTM containing 3% sucrose and various concentrations of IBA. Individual shoots were transferred after 5 days to the same medium containing 0.6% agar without IBA, or to filter paper bridges over half-strength MSH or BTM with 3% sucrose and no hormones.

Plantlets were placed in pots containing a vermiculite and perlite mix (1:1 v/v) and watered with half-strength BTM salts. Potted plantlets were placed in flats over a tray of water and covered with clear plastic propagation domes. Plantlets were kept under 60 $\mu E/sec/m^2$ of mixed cool-white and Sylvania GroLux lamps with a 14-hr photoperiod at 23° C. Each week the plastic domes were changed with ones containing an increasing number (6, 12, 24, or 48) of equally spaced 6-mm diameter holes to gradually reduce the humidity to ambient levels. Plantlets were watered as needed with sterile, half-strength BTM salts until well established and placed in a greenhouse.

RESULTS AND DISCUSSION

Shoot Production and Subculture

Compos tion of the medium and the presence of agar affected explant competence and the quality of primary shoots. Competency was more than twice as great on agar medium than in liquid culture (Table 1). However, shoot quality was often poor on agar media. Fifty percent or more of the primary shoots were abnormal with thick stems, unexpanded leaves and necrotic stipules. Primary shoots produced in liquid culture had thin, often red-pigmented stems and expanded leaves with no evidence of necrosis (Fig.1). Two to three primary shoots were produced per competent explant in 4 wk. (Table 1).

We found that the average number of primary shoots produced was not a sufficient way to compare the effects of medium composition on production of quality shoots. Many small shoots $\leq$1.0 cm long died when excised for subculture or rooting or failed to elongate when dominant shoots were removed from the explants. Therefore, a weighted shoot index based on shoot lengths and numbers was calculated for each competent explant (Table 1). Average shoot index was greatest for primary shoots produced on explants on BTM with agar. This medium also produced the fewest abnormal shoots.

Table 1. Effects of three tissue culture media and the presence of agar on primary shoot production by pin oak explants.

Media[z]	% Competent Explants	Growth parameters for competent explants[y] No. of shoots	Shoot index[x]	% Abnormal shoots
Agar (0.6%)				
Broadleaf tree	96	3.2[a]	6.3[a]	19
Woody plant	100	2.6[a]	4.8[b]	52
Modified Schenck & Hildebrandt	84	1.8[a]	3.1[c]	29
Liquid				
Broadleaf tree	42	1.6[a]	2.9[a]	0
Woody plant	37	2.3[a]	4.0[a]	0
Modified Schenck & Hildebrandt	44	1.9[a]	3.2[a]	0

[z] All media contain 3% sucrose, 8.9 uM BAP, pH 5.3.
[y] Mean separation in columns for agar or liquid media by Duncan's multiple range test, P = 5%. Growth parameters rated at 4 wk. N = 30-44.
[x] Shoot index per explant = shoot length factor x no. of shoots. Shoot length factors and corresponding shoot lengths were: 1 = 0.5 - 0.9 cm; 2 = 1.0 - 1.9 cm; 3 = 2.0 - 2.9 cm; 4 = 3.0 - 3.9 cm; 5 = 4.0 cm and >.

Several experiments were done to determime the effects of benzylaminopurine (BAP) concentration and presence of agar on primary shoot production. When BAP concentrations of 8.9, 0.89 and 0.089 µM were compared in BTM agar culture (14 ml, 0.6% agar), explant compentency was unafffected, and the percent abnormal shoots tended to decrease with decreasing BAP concentration. The trend in shoot quality was quite variable among several experiments on agar BTM and may be related to other factors such as physiological age and condition of the initial explants. Mean number of shoots per explant (about 2.4) was the same for 8.9 and 0.89 µM BAP but decreased to 1.1 primary shoots per explant at 0.089 µM BAP. Shoot index was greater at 0.89 µM BAP than at 8.9 or 0.089 uM within an experiment, but the absolute values for shoot indices tended to vary among experiments. When 3 ml liquid cultures (BTM, 8.9 µM BAP) were compared to 3 ml agar cultures on the same medium, the only difference was in explant competence; 22% in liquid and 100% in agar cultures. No abnormal shoots occurred in either 3 ml liquid or 3 ml agar cultures and primary shoots numbers (2.4) and shoot indices (4.4) were not significantly different. It appears that both BAP concentration and presence of agar can effect primary shoot numbers and quality, and that agar in some way strongly affects explant competence. The varying effects of agar or other gel matrices on growth parameters in tissue culture have been described, but the cause(s) are not easily explained (Chun et al., 1986; Lee et al., 1986).

Pin oak shoots could be subcultured easily in liquid medium (BTM or MSH) in GA-7 vessels. A BAP concentration of 0.89 µM was found best because higher concentrations caused the production of many buds and short shoots that did not develop further when subcultured. Subcultured primary shoots produced an average of 1.7 secondary shoots from axillary buds after 2 to 3 weeks. Initial explants with primary shoots removed produced about three new primary shoots after 2 weeks and produced two or three additional flushes over 4 to 6 weeks before they declined and died. Competency of shoots and initial explants ranged from 81 to 93% in liquid subculture. Abnormal shoot development (Fig.1) did not occur in liqid subculture, and abnormal primary shoots from agar culture produced normal secondary shoots from axillary buds after transfer to liquid subculture. Pin oak shoots have been carried successfully through four subcultures in liquid medium with production of rootable shoots.

Results over three years suggest that it will be possible to establish in vitro shoot cultures with explants from mature trees. Softwood shoots forced from dormant branches yielded explants with $\leq$ 20% contamination. Explant competence on agar ranged from 0-50%, depended on the source tree and varied from year to year from the same tree. The results suggest that research on auxin/cytokinin ratio and time of sampling during the dormant season are needed.

In Vitro Rooting

Subcultured pin oak shoots were rooted successfully by all three IBA treatments. There was no significant difference ($P = 0.05$) in percent rooting (44-62%) or in number of roots per shoot (3 or 4) after 3 wk between the dip treatment or placing excised shoots on agar medium containing IBA. A post-treatment dark period of up to 2 wk was not beneficial to rooting. Toxicity symptoms often occured after the dip treatment. Treatment by the method modified from Zimmerman and Fordham (1985) gave similar rooting percentages and roots per shoot as the other treatments after 3 wk on agar medium, and 70-90% rooting has been obtained consistently 6-7 wk after treatment. IBA concentrations of 25, 75 or 123 µM gave no sifnificant differences in rooting percent. Numerous attempts to transfer rooted shoots from agar media to various potting mixes were unsuccessful. Examination showed that the roots lacked root hairs which probably prevented plantlet establishment.

Vigorous root systems with abundant root hairs were produced when shoots treated with 123 µM IBA by the modified Zimmerman method were placed on filter paper bridges over liquid medium (Fig.2) . Eighty percent or more of the shoots rooted within 2 to 3 weeks.

Several attempts to root IBA-treated shoots by the method described by Bennett and Davies (1986) with Jiffy-7 pellets (Jiffy Prod. Ltd.) or vermiculite were unsuccessful. Shoots became necrotic within 2 wk after IBA treatment, and root development did not occur.

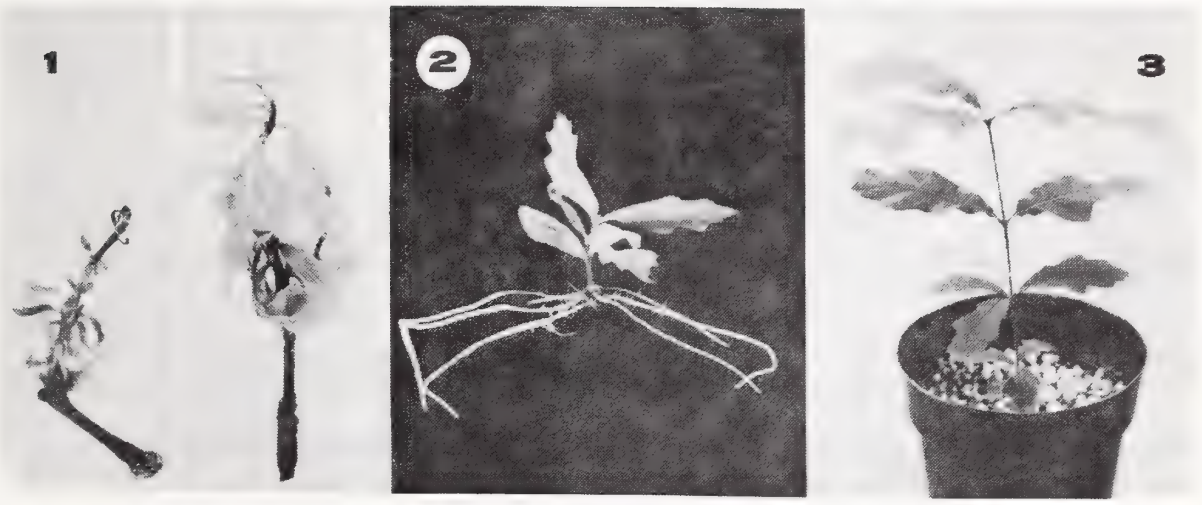

Fig. 1. Primary shoots produced after 4 wk in culture. Abnormal shoot from agar culture (left), normal shoot from liquid culture (right).
Fig. 2. Pin oak plantlet rooted on a filter paper bridge over half-strength BTM.
Fig. 3. In vitro propagated pin oak acclimatized to greenhouse conditions.

Acclimatization

In repeated trials, 80 to 93% of rooted plantlets were successfully adapted to greenhouse conditions by the procedures described. During the 5- to 6-wk acclimatization period most plantlets produced two or three new growth flushes (Fig.3). No morphological differences have been noticed between micropropagated plants and seedlings grown from acorns.

SUMMARY

Pin oak was in vitro propagated from seedling explants. Broadleaf tree medium gave the best results of those tested. Agar greatly affected explant competence, and both agar and BAP affected shoot quality. Although an optimum concentration was not determined, 0.89 - 8.9 µM BAP in agar medium would strike a balance between high competence and good shoot quality. Shoot subcultures were maintained in liquid medium with 0.89 µM BAP. Subultures yielded high quality shoots and appeared to be a convenient way to clonally propagate pin oak. Several procedures for IBA treatment were adequate for

root initiation but good root hair development, as occurred on filter paper bridges, was required for plantlet establishment. Plantlets were readily acclimatized to greenhouse conditions if potted in a well-drained medium.

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ENHANCEMENT OF SHOOT ORGANOGENESIS IN CONIFERS

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Abstract. Successful genetic modification of conifers through gene transfer and transgenic plant production is dependent upon effective plant regeneration systems. Plantlets from a large number of conifer species have been successfully propagated via organogenesis using cotyledon explants and they may be potenially amenable to gene transfer technology. However, the primary limitation for cotyledonary explants is the low number of regenerated plantlets and the long timespan between initiation and plantlet formation. Shoot production can be enhanced by providing more optimal hormone conditions in the shoot induction medium. Abscisic acid (ABA), in addition to auxins and cytokinins, enhances shoot formation from conifer explants. ABA enhances the morphogenic area of cotyledon explants from two *Pinus* species. The degree of enhancement is related to the seed source and the length of exposure. Shoot production is completely blocked by fluridone (FLUR), an inhibitor of endogenous ABA synthesis. Abscisic acid appears to be an important hormone in shoot morphogenesis.

Keywords: ABA: Abscisic acid, BA: 6-Benzylaminopurine, cotyledon explants, FLUR: fluridone, GD: Gresshoff and Doy (1972) nutrient medium, NAA: α-Naphthaleneacetic acid, *Pinus taeda* L., *Pinus virginiana* Mill., Shoot primordia.

INTRODUCTION

Successful genetic modification of conifers through gene transfer and transgenic plant production is dependent upon effective plant regeneration systems (Sederoff et al. 1987). Plantlets from a large number of conifer species have been successfully propagated via organogenesis using various explants (Campbell and Durzan 1975, Sommer et al. 1975, Mott et al. 1977, Reilly and Washer 1977, Mehra-Palta et al. 1978, Karnosky 1981, Kaul and Kochhar 1985, Patel and Thorpe 1984, 1986, Gladfelter and Phillips 1987, Perez-Bermudez and Sommer 1987, Kaul 1987, Jain et al. 1988a). Cotyledon explants in conifers appear to be amenable to gene transfer technology.

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However, the primary limitation for cotyledonary explants is the low number of regenerated plantlets and the long timespan between initiation and plantlet formation. Larger number of shoots produced per cotyledon explant in a shorter period of time will give a better opportunity for producing a large number of genetically modified propagules after gene transfer.

Shoot production could be enhanced perhaps by providing more optimal hormonal conditions in the shoot induction medium. In addition to auxins and cytokinins (Mott and Amerson, 1981), another plant hormone such as abscisic acid (ABA) may increase shoot production from conifer explants. Abscisic acid could possibly have such a role because previous studies have indicated that it is associated with embryo maturation and development. For example, ABA has been shown to: 1) increase the accumulation of storage proteins in developing embryos of *Glycine* (Bray and Beachy 1985), *Brassica* (Crouch et al. 1985), *Gossypium* (Galau et al. 1986) and *Triticum* (Willamson et al. 1985); 2) stimulate growth of zygotic seeds in *Glycine* (Ackerson 1984) and *Zea* (Fong et al. 1983); 3) influence normal somatic embryo development in *Picea* (Becwar et al. 1987, von Arnold and Hakman 1988, Jain et al.1988b) and *Daucus* (Kamada and Harada 1981); and 4) induce embryogenesis in *Carum* (Ammirato 1974) and to enhance somatic embryogenesis from leaf explants in *Pennisetum* (Rajasekaran et al., 1987).

Abscisic acid also appears to influence morphogenesis. Root formation as well as root growth was increased in *Zea* callus (Abou-Mandour and Hartung, 1986). In this paper, we show that ABA enhances shoot production from pine cotyledon explants and that shoot production is blocked by fluridone (FLUR), an inhibitor of ABA synthesis via carotenoid metabolism (Fong et al. 1983).

MATERIALS AND METHODS

The first experiment was performed with half-sib seeds of *Pinus taeda* L. (Texas Forest Service BA3R13-41 x Open, collected in 1983) from Bastrop, Texas. Seeds were surface sterilized with H_2O_2 for 20 min, washed in tap water for 24 h and pretreated 5 to 6 d in 1% H_2O_2 at room temperature (22°C) to stimulate germination (Ching and Parker 1958). Seeds were again surface sterilized with 15% (v/v) clorox (5.25% sodium hypochlorite) for 5 min followed by 3 rinses of sterile water. Embryos were aseptically removed from the seeds, and the cotyledons were excised and transferred to GD (Gresshoff and Doy 1972) nutrient agar medium supplemented with BA (44 μM) and NAA (0.05 μM) before autoclaving. Abscisic acid (10^{-7}M) or FLUR (50 mg l^{-1}) was filter sterilized prior to its addition to the autoclaved medium.

Cotyledon explants (5 to 9) from 25 seeds for each treatment were placed in 5 Petri dishes containing nutrient agar as described above. Explants from 5 seeds were placed in each dish and incubated under continuous light (250 μmol $m^{-2}s^{-1}$) at 25°C. After 3 weeks of incubation the cotyledons were observed with light microscopy and scored for the percentage of cotyledon area per seed having shoot primordia which indicated shoot

initiation. Each seed (5 to 9 cotyledons) was regarded as a whole (100%) and the percent area having shoot primordia on individual cotyledons was scored (e.g., when an individual seed has 5 cotyledons, each cotyledon is 20%; 5 cotyledons/seed X 20%/cotyledon = 100% seed). Scoring was done for all 25 seeds at 4 weeks. For FLUR-treated cotyledons, the fresh weight after treatment was determined.

In a second experiment, cotyledons were exposed to ABA or FLUR for 4 weeks, scored for shoot initiation and then transferred to 0.5-strength GD medium (without NAA, BA or ABA) for 4 more weeks for shoot elongation (Mott and Amerson 1981). Controls were scored and transferred at the same time. The number of shoots which were 2 mm or greater in height were then counted and the totals compared between ABA-treated cotyledons and the controls.

In a third experiment with cotyledon explants of *Pinus virginiana* Mill. obtained from the Texas Forest Service, ABA (7.6 μM) was added to a full strength GD agar medium supplemented with BA (22.20 μM) and NAA (0.05 μM). The control treatment was the same medium without the ABA. All explants were obtained from seeds germinated in 1% H_2O_2 for 7 days. The control treatment consisted of 72 cotyledon explants with 24 inoculated in each of 3 Petri dishes. The ABA treatment consisted of 120 cotyledon explants inoculated in groups of 24 in 5 Petri dishes. Dormant cotyledons with no evidence of shoot meristem activity were discarded.

RESULTS AND DISCUSSION

In our first experiment with seeds originating from Bastrop, Texas the cotyledon explants showed typical development after 4 weeks on the control medium (GD+BA+NAA). The cotyledons had a distinctive green color and showed a very bumpy appearance indicating cell proliferation at their surfaces. When ABA was present in the medium (GD+BA+NAA+ABA), the cotyledons greatly increased in size and they had a bright green color. The regions of cell proliferation appeared similar to those seen in control cotyledons, differing only in that there was an increased area with shoot primordia in 10^{-7}M ABA-treated tissues (Table 1). The percent of total cotyledon surface area with shoot primordia was assessed after 4 weeks. ABA-treated cotyledon explants had significantly larger surface areas with shoot primordia than the control explants.

Because 10^{-7}M ABA was shown to be effective in enhancing morphogenesis in our preliminary experiments, this concentration of ABA was used. Other workers had shown that it takes 4 weeks for *Pinus* cotyledon explants to respond fully to BA+NAA for shoot initiation (Mott and Amerson 1981), and it appeared from our preliminary studies that a prolonged treatment with ABA did not increase the response significantly. Therefore, the effect of ABA was compared to controls after 4 weeks of treatment.

Table 1. Enhancement of shoot production by ABA from *Pinus taeda* cotyledon explants

Concentration* of ABA (μM)	Percent of Cotyledon Surface Area with Shoot Primordia (%)
0	17.7
0.1	37.4*

*BA = 10 mg l^{-1}, NAA = 0.1 mg l^{-1}
**Significant at the 0.05 level

Endogenous ABA synthesis is inhibitied by FLUR via inhibition of carotenoid synthesis (Fong et al. 1983). Fluridone-treated cotyledons showed some very small proliferations of growth on the surface and they were white in color. There was no measureable increase in fresh weight (Table 2). The fresh weight of the cotyledon explants at the time of inoculation was 1 mg. After 4 weeks of FLUR treatment, the fresh weight remained at 1 mg, indicating no growth or shoot production (Table 2).

Table 2. Fresh weight of cotyledon explants of loblolly pine (*Pinus taeda* L.) after treatment with FLUR for 4 weeks.

Concentration (mg l^{-1})	Fresh Weight (mg)
0	15
50	1

In our second experiment, the objective was to determine the effect of ABA on the number of shoots produced. Because the shoots were at various developmental stages, only those shoots that were 2 mm or more in height after being subjected to the shoot elongation treatment for 8 weeks were counted (Table 3). A 56% increase in number of shoots produced from Bastrop cotyledon explants was observed when they had been exposed to 10^{-7}M ABA for 4 weeks. No shoots were produced on cotyledons treated with FLUR. After 4 weeks on elongation medium there was no evidence of shoot production, whereas many shoots were present on the control cotyledons. These data indicate that FLUR completely blocked shoot production.

Table 3. Enhancement of shoot production by ABA from *Pinus taeda* cotyledon explants after 8 weeks on shoot elongation media*

Concentration of ABA (μM)	No. of Shoots ≥ 2mm	% Increase
0	225	
0.1	522	56

*(Mott and Amerson, 1981)

We have also observed an ABA-enhanced increase in surface area with shoot primordia on cotyledon explants of other *Pinus* species. In *Pinus virginiana*, we observed a 65% increase in shoot primordia surface area of cotyledon explants after 4 weeks of ABA exposure (Table 4).

Table 4. Enhancement of shoot primordia production by ABA from *Pinus virginiana* cotyledon explants

ABA Concentration* (μM)	Number of Cotyledons Observed	Percent of Cotyledon Surface Area with Shoot Primordia (%)
0	53	26.3
7.6	68	43.1**

*BA = 22.2 μM, NAA = 0.05 μM.
**Significant at 0.001 level

The shoot response to ABA appears to be dependent upon the length of exposure time as well as the seed source. Exposures longer than four weeks appeared to halt shoot development, indicating that higher than optimal levels of ABA may be present in the tissue. This may also explain why ABA addition to callus in both *Pinus strobus* and *Pinus echinata* showed no increase in frequency of somatic embryogenesis or organogenesis (Kaul and Kochhar 1985). Different seed source responses to exogenous ABA may be due to different levels of endogenous ABA in explant tissues. As expected for a hormone mediated event, there is an optimal ABA concentration for shoot enhancement with more effectiveness at 10^{-7}M than at either 10^{-6}M or 10^{-8}M for loblolly pine. Similar optimum ABA concentration dependent responses were reported for callus generated from *Pinus spp.* (Kaul and Kochhar, 1985).

At this time, it is not clear whether ABA enhances shoot production primarily by increasing shoot induction or shoot maturation and development, or whether it is increasing both. Our studies with FLUR, an inhibitor of

ABA biosynthesis (Fong et al. 1983), showed a complete inhibition of shoot initiation in loblolly pine cotyledon explants, suggesting that ABA may have a role in shoot induction (Ackerson 1984). Furthermore, Bastrop explants produced significantly more shoots than the controls when treated with ABA, which may indicate shoot induction. On the other hand, studies with other plant systems such as *Glycine* (Bray and Beachy 1985), *Brassica* (Crouch et al. 1985), *Gossypium* (Galau et al. 1986), *Triticum* (Willamson et al. 1985), *Picea* (Becwar et al. 1987, Jain et al. 1988) and *Daucus* (Kamada and Harada 1981) all suggest that ABA has a role in tissue maturation and development.

Environmental stresses have been shown to increase ABA levels in stressed plant tissues. Stresses such as osmotic shock (Jain et al. 1988b), water stress (Liu and Lai 1985) and salt stress (Litz 1986), all stimulate somatic embryo development in embryogenic culture. Osmotic stress increased root formation from *Zea* callus (Abou-Mandour and Hartung, 1986). Water stress has been shown to induce transcriptional cellular processes leading to the production of ABA (Guerrero and Mullet, 1986) suggesting that ABA synthesis in response to stress may lead to subsequent tissue development in morphogenesis. In addition to embryogenesis, the present study shows for the first time that ABA also has a role in *Pinus taeda* organogenesis. Conifer explant tissues seem to be responsive to ABA, because we have observed similar responses in two pine species.

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SOMACLONAL VARIATION IN EASTERN COTTONWOOD FOR RESISTANCE TO LEAF RUST DISEASE

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Abstract.--Adventitiously regenerated plants of Populus deltoides (somaclones), derived from leaf callus, were screened for leaf rust reaction to two races of Melampsora medusae using a leaf assay technique. Most of the somaclones exhibited disease reactions similar to the parent but some were either more resistant or more susceptible than the parent. Somaclonal variation for leaf rust resistance was race-specific in nature as we observed a significant somaclone x race interaction. Complete resistance was not observed in any of the somaclones but a few somaclones exhibited a longer latent period and reduced sporulation when compared with the parent. If field studies confirm these laboratory observations, then the somaclonal variation may be an useful approach to increase the partial resistance in elite lines of eastern cottonwood to leaf rust disease.

CONCURRENT SESSION: 2A

PREPARATION OF CELL-WALL-FREE PROTOPLASTS FROM THE CHESTNUT BLIGHT FUNGUS

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Abstract.--Certain isolates of Cryphonectria parasitica (Murr.) Barr (=Endothia parasitica (Murr.) P. J. & H. W. Anderson) contain cytoplasmically transmitted dsRNA which is believed to render mycelium hypovirulent. Practical utilization of hypovirulence in fungi is limited because of the general presence of mating incompatibility factors. A method of forcing vegetative fusion, and hence possible transfection of the dsRNA, involves use of electromanipulation. This research reports the protocol necessary to produce cell-wall-free protoplasts. Two strains were used. E7 is white in culture and is a hypovirulent strain which produces large titers of dsRNA. Strain 591 is virulent, is dsRNA free, and produces a yellowish-brown culture in Liquid Complete Medium (LCM) (Phytopathology 67:1393- 396). Two mm dia agar plugs of hyphal tips were removed from a 4-day-old culture on solid LCM and placed on a special cellophane membrane (6 x 6 cm) appressed to solid LCM (in 1% agar) and incubated in the dark for 2 days at 27°C. The cellophane pieces, each with visible growth, were then floated on LCM and grown at room temp for 48 hr under a 16 hr photoperiod. Membranes with colonies were then placed on one-half strength LCM for 24 hr, then washed with 3 changes of 0.5 M MgSO4 in distilled water.

In order to remove the cell walls, mycelia were carefully removed intact from the cellophane membrane and resuspended in 15 ml of 0.5 M MgSO4 with 1 mg/ml of commercial wall-lytic enzyme and 1mM CaCl2 and incubated for 16 hr at 24°C. Protoplasts were collected by serial centrifugation and resuspended in MCT medium (0.5 M mannitol, 1mM CaCl2, and 50 µg/ml wall-lytic enzyme). Three washes were done using centrifugal sedimentation, changing the MCT medium each time. The final protoplast pellet was suspended in MCT medium and adjusted to 1 x 10 protoplasts/ml for electromanipulation. The advantage of this procedure over liquid culture was the avoidance of tedious and time consuming filtration and washing.

Results showed that the cell walls of the E7 isolate were easy to remove enzymatically. E7 yielded approximately 10 times as many protoplasts as did 591 under identical growth conditions. Protoplasts from 591, however, were larger in overall size and had larger vacuoles than did E7. The cytoplasm of E7 appeared much denser.

FACTORS REGULATING LOBLOLLY PINE (Pinus taeda L.) SOMATIC EMBRYO DEVELOPMENT

M.R. Becwar[1/] and R.P. Feirer[2/]

Abstract. The effect of medium components and culture conditions on somatic embryo development and the level of storage lipids (triglycerides) was studied in embryogenic callus of loblolly pine. A factorial experimental design was employed to test the effects of three factors on somatic embryo development: 1) basal medium, DCR (Gupta and Durzan, 1985) and MSG (Becwar et al., 1988); 2) culture condition, light versus dark; and 3) level of ABA. After 28 days on the treatments embryogenic calli were collected for somatic embryo counting and determination of triglyceride levels. Basal medium had a significant effect on the number of somatic embryos per gram of callus. Similarly, the level of ABA significantly affected somatic embryo development. Averaged across light and dark treatments, MSG medium with 30 µM ABA produced about 600 total somatic embryos per gram of callus and 15% of these reached stage 2 of embryo development (Hakman and von Arnold, 1988). Basal medium and ABA also influenced the level of triglycerides. The highest level of triglycerides accumulation, about 15 µg/mg protein, occurred on the MSG medium containing 10 µM ABA. Light versus dark culture conditions did not significantly affect the measured parameters. These results suggest that the beneficial effect of ABA on somatic embryo development in loblolly pine may in part be related to ABA-enhanced accumulation of lipids. This ABA effect is strongly influenced by the basal medium on which the cultures are grown.

Keywords: Pinus taeda L., somatic embryogenesis, abscisic acid, triglycerides

INTRODUCTION

Although embryogenic callus of loblolly pine can be initiated reproducibly, development of the resulting immature somatic embryos remains difficult. Treatment with abscisic acid (ABA) has recently been reported to enhance development of somatic embryos of Norway spruce and white spruce (von Arnold and Hakman, 1988; Dunstan et al., 1988). ABA has also been shown to induce the accumulation of storage lipids in somatic embryos of Norway spruce (Feirer, et al., in press). Preliminary experiments in our laboratory have demonstrated the positive effects of ABA on the development of loblolly pine somatic embryos (Becwar et al., in press). These studies suggested that the transfer of embryogenic calli to MSG basal medium (Becwar et al., in press), sucrose levels of 3 to 6%, and 10 µM ABA promoted embryo development.

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The objectives of the study described here were to optimize conditions for maturation of somatic LP embryos. The experimental design employed in this study afforded the opportunity to obtain quantitative data and statistical evidence for the effect of several factors on somatic embryo development and levels of storage lipids. Variables tested included basal medium, ABA treatments and the presence or absence of light.

MATERIALS AND METHODS

Embryogenic callus of loblolly pine (Pinus taeda L.) was initiated from an immature embryo explant collected August 3, 1987 (clone #7-34; see Becwar et al., in press). The callus was initiated and maintained on DCR basal medium (Gupta and Durzan, 1985) containing 2,4-D and 6-benzylaminopurine (3 mg/l and 0.5 mg/l, respectively), hereafter referred to as DCR 3/0.5 medium. The embryogenic callus was maintained in the dark at 23 °C and subcultured every two weeks.

The factorial design employed tested the effects of three factors on somatic embryo development: 1) basal media (DCR and MSG, both containing 3% sucrose). MSG medium was formulated/described by Becwar et al.(in press). 2) levels of ABA (0, 1, 10, 30 μM). and 3) culture conditions (light and dark). Light intensity was 80 μEm^{-2} sec^{-1} from cool-white fluorescent and 20 μEm^{-2} sec^{-1} from incandescent bulbs. Three embryogenic calli (100 mg each) were transferred to each culture plate which contained 10 ml of one of the above 16 treatments. Each treatment was replicated 4 times (4 plates). After 28 days of growth on these developmental media, embryogenic calli were collected for somatic embryo counting and determination of triglyceride levels.

To determine the number of somatic embryos per callus, a modification of the technique described by Becwar et al. (1987) was used. Each piece of callus was weighed and added to 1 ml of water in a 10 cm test tube, which was stoppered and vigorously agitated for 10 sec. One ml of 1.2 % low melting point agarose containing 1 % Merthiolate, which had been tempered to 40 °C, was added to the dispersed callus in the test-tube. After mixing, the 2 ml suspension was poured onto a 10 ml base layer of 0.8% agarose in 100 mm plastic petri plates. After cooling, the somatic embryos were counted using a dissecting microscope at 15X magnification. The plates were placed on a background grid to facilitate counting. Both the total number of somatic embryos and the number of "large" well-formed embryos having dense, smooth-surfaced embryonal heads (stage two according to the description of Hakman and von Arnold, 1988) were counted.

Triglycerides were quantified by biochemical analysis. A method used to determine triglyceride levels in human serum, commercially available in kit form, was adapted for use with plant tissues (Feirer et al., 1989).

Data were analyzed by factorial ANOVA, this approach being useful for identifying the variables within a complex, multifactorial experiment which significantly affect culture response.

RESULTS AND DISCUSSION

The effects of the media components and light on the production of loblolly pine somatic embryos appear in Figure 1. To illustrate statistical results, abbreviated ANOVA tables are also included (Table1). Both the basal medium and ABA treatments significantly affected total embryo production in the calli. MSG medium led to greater embryo production than DCR. The enhancement of embryo production by ABA was also evident in both basal media, with optimal ABA concentrations being 10 to 30 μM, perhaps higher. This is within the range previously reported to augment somatic embryo formation in Norway spruce

Figure 1. Effect of ABA, basal medium and light on somatic embryo production in loblolly pine. The total number of somatic embryos per gram of callus was determined.

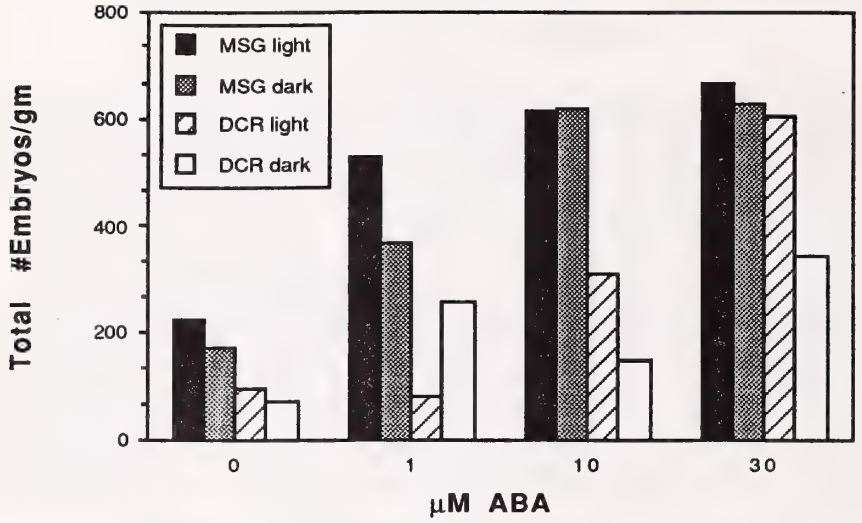

Table 1. Analysis of variance of the effect of media, light and ABA on total production of loblolly pine somatic embryos.

SOURCE	DF	MS	F	Prob.	
Treatment	15	192296	6.57	< 0.05	*
Media	1	891542	30.49	0.000	*
Light	1	65712	2.25	0.140	ns
ABA	3	507073	17.34	0.000	*
media x light	1	179	0.01	0.938	ns
media x ABA	3	57638	1.97	0.131	ns
light x ABA	3	17218	0.59	0.626	ns
media x light x ABA	3	60411	2.07	0.117	ns
Error	47	29236			
Total	62				

* = significant
ns = nonsignificant

and white spruce (von Arnold and Hakman, 1988; Dunstan et al., 1988). The effect of light (presence or complete absence) on embryo production was not significant (Table 1).

While a simple count of all somatic embryos within a callus is representative of embryo initiation and early development, it may not be relevant to or accurately reflect the degree of development or maturation of the somatic embryos. In order to better determine the effects of culture parameters specifically on somatic embryo development, a subpopulation of the embryos was counted. "Large", well formed, smooth surfaced embryos were counted to determine the production of further developed somatic embryos by the calli. In this case, the effects of basal medium were more pronounced (Figure 2, Table 2). Of the parameters tested, basal medium had the greatest effect on embryo development. MSG medium was clearly superior to the DCR formulation used. The effect of ABA on embryo development was evident, especially when used in conjunction with MSG basal medium (Figure 2). It again appeared that the optimal ABA concentration was 30 µM or higher. Obviously, future experiments must use concentrations above 30 µM in order to ensure that the optimal ABA response is being attained. ABA additions to DCR medium did not enhance development. As was found for total embryo production (Figure 1), light also failed to influence somatic embryo development (Figure 2).

ABA has been shown to enhance the development of somatic embryos of a number of plant species. The maturation of angiosperm somatic embryos to more advanced stages of development is promoted by ABA, and more importantly, the treated embryos appear to have a more normal morphology (Ammirato, 1974, 1983). The enhancement of conifer somatic embryo development by ABA has been reported in a number of recent publications. Development of both Norway spruce and white spruce somatic embryos was improved by treatment of cultures with ABA (Becwar et al., 1987; Dunstan, et al., 1988; Feirer et al., in press; von Arnold and Hakman, 1988). Our results show that ABA similarly improves the development of loblolly pine somatic embryos. It appears, then, that future studies on the promotion of loblolly pine somatic embryo development should include ABA in the MSG basal medium as a starting point or a baseline against which to judge and test the effects of other variables. At this point, the effect of light appears to be insignificant, but experiments having photoperiods and light quality as variables cannot be dismissed and should be considered.

The effects of culture variables on triglycerides, a biochemical parameter theorized to be important to the normal development of conifer embryos (Feirer et al., 1989), were also determined. Once again, basal media and ABA significantly influenced the response. Tissues grown on MSG contained significantly higher levels of triglycerides than those grown on DCR (Figure 3, Table 3). Triglyceride accumulation was affected by ABA treatments, optimal triglyceride accumulation being at (or near)10 µM ABA. Light had no effect on triglyceride levels in these tissues.

In their report describing the enhancement of morphological development of Norway spruce somatic embryos by ABA, von Arnold and Hakman (1988) noted that intracellular lipids were also affected. Using a histochemical stain specific for lipids, more of these reserve compounds were observed in the ABA-treated tissues. This finding complements and corroborates our biochemical results on the enhancement of triglyceride accumulation by ABA. Clearly, then, ABA promotes the accumulation of reserve lipids. These findings are consistent with the ability of ABA to suppress precocious germination, allowing the formation of embryo-specific storage proteins (Finkelstein et al., 1985; Quatrano, 1986). Low levels of storage or reserve compounds may be characteristic of somatic embryos of many plant species. In addition to

Figure 2. Effect of ABA, basal medium and light on somatic embryo maturation in loblolly pine. The number of "large" well formed somatic embryos having smooth, dense embryonal heads per gram of callus was determined.

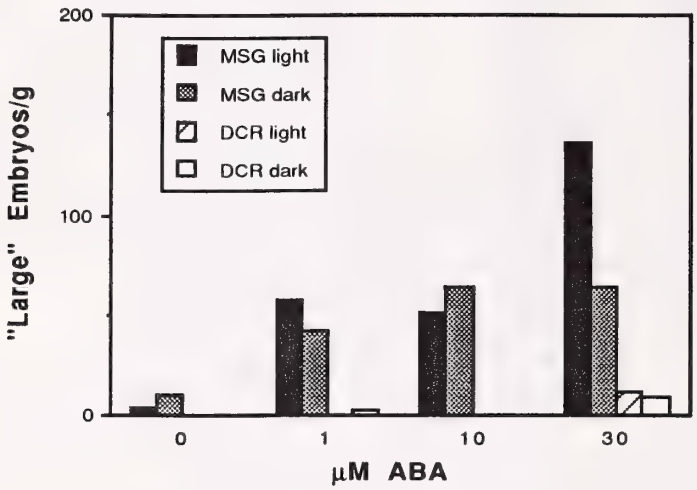

Table 2. Analysis of variance of the effect of media, light and ABA on production of "large" loblolly pine somatic embryos.

SOURCE	DF	MS	F	Prob.	
Treatment	15	5861	2.07	< 0.05	*
Media	1	40054	14.17	0.000	*
Light	1	1220	0.43	0.514	ns
ABA	3	7244	2.56	0.066	
media x light	1	1255	0.44	0.508	ns
media x ABA	3	4745	1.68	0.184	ns
light x ABA	3	1638	0.58	0.631	ns
media x light x ABA	3	1501	0.53	0.663	ns
Error	47	2826			
Total	62				

* = significant
ns = nonsignificant

Figure 3. Effect of ABA, basal medium and light on triglyceride levels in cultured loblolly pine tissues.

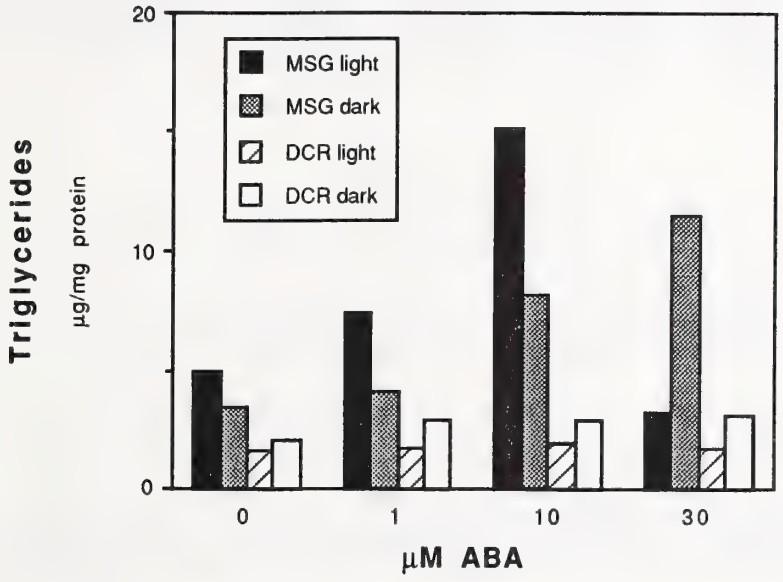

Table 3. Analysis of variance of the effect of media, light and ABA on loblolly pine triglycerides

SOURCE	DF	MS	F	Prob.	
Treatment	15	61.87	7.66	.0001	*
Media	1	401.40	49.67	.0001	*
Light	1	0.07	.009	0.927	ns
ABA	3	48.01	5.94	0.002	*
media x light	1	14.41	1.78	0.188	ns
media x ABA	3	36.92	4.57	0.007	*
light x ABA	3	45.88	5.68	0.002	*
med. x L x ABA	3	39.95	4.94	0.005	*
Error	48	8.081			
Total	63				

* = significant
ns = nonsignificant

the low levels of reserve lipids (triglycerides) in cultured conifer tissues, somatic embryos of rapeseed and cotton have been reported to accumulate storage proteins at reduced levels (Crouch, 1982; Shoemaker et al., 1987). This failure to accumulate adequate storage reserves may hinder continued development and germination of somatic embryos. The beneficial effect of ABA on somatic embryo development may then be related to the ABA-enhanced accumulation of storage compounds.

Since a good correlation exists between triglyceride content and somatic embryo development (compare Figures 2 and 3), studies attempting to further enhance the triglyceride content will continue. The role of ABA in this process will continue to be explored, along with other means to improve triglyceride biosynthesis and accumulation.

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OPTIMIZATION OF THE YELLOW-POPLAR EMBRYOGENIC SYSTEM

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Abstract.--Experiments directed at improving the efficiency of yellow-poplar (Liriodendron tulipifera) somatic embryogenesis have focused on (1) Increasing the percentage of yellow-poplar cultures that become embryogenic and (2) Increasing the percentage of somatic embryos that are well-formed and capable of conversion to plantlets. To raise the percentage of embryogenic cultures, we have concentrated on defining the optimal stage of zygotic embryo explant using morphological and biochemical developmental markers. Yellow-poplar cultures were initiated from developing zygotic embryos from seeds collected every two weeks from 4 weeks post-pollination (June 10) until seed maturity (September 14). Potential of an explant to produce an embryogenic culture peaked during the 8th week following pollination, with over 25 percent of the explants producing embryogenic callus in most full-sib families. Production of cultures with embryogenic callus declined to near zero for mature zygotic embryos. The maximum embryogenic potential for an explanted zygotic embryo corresponded to the globular to early heart stages of embryo development. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of zygotic embryos from 5 sampling dates showed that decline of embryogenic potential appeared to be correlated with an increase in the level of a protein of approximately 55 kD, and some qualitative changes in polypeptides. To promote the production of well-formed somatic embryos capable of conversion to plantlets, we tested physical and chemical treatments on embryogenic suspension cultures. Proembryogenic masses removed from auxin-supplemented medium and placed in hormone-free medium generally produced malformed and clustered somatic embryos that were asynchronous in their development and that germinated precociously. Production of synchronous, mature, well-formed embryos was promoted by fractionating embryogenic suspensions on sieves and culturing in a medium supplemented with 5×10^{-7} M abscisic acid (ABA). However, conversion of the embryos was inhibited once they were transferred to solid medium, possibly due to the effects of residual ABA.

Keywords: Liriodendron tulipifera, somatic embryogenesis, SDS-polyacrylamide gel electrophoresis, abscisic acid.

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INTRODUCTION

Yellow-poplar (Liriodendron tulipifera L.) probably attains the greatest height of any broad-leaved tree in eastern North America and may also reach the largest diameter (Harlow et al. 1979). The species, characterized by straight form, rapid growth and wood of exceptional working quality (Wilcox and Taft 1969) is considered to be one of the most important hardwoods in the United States. Our first report of somatic embryogenesis from yellow-poplar tissue cultures was based on four embryogenic lines derived from immature zygotic embryo explants from seeds of a single mother tree (Merkle and Sommer 1986). Explants produced a fast-growing, pale yellow, nodular callus following 1-2 months culture on a modified Blaydes' (Witham et al. 1971) conditioning medium supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 0.25 mg/l 6-benzyladenine (6BA) and 1 g/l casein hydrolysate (CH). Within 1 month following transfer of embryogenic callus to a hormone-free induction medium, somatic embryos differentiated. A very low percentage of embryos began to convert spontaneously on induction medium and when moved to a Risser and White's (1964) medium (RW), would develop into plantlets. Over the past two years, we have moved hundreds of somatic embryo-derived plantlets from in vitro conditions, acclimatized them in a controlled humidity chamber, transferred them to the greenhouse and from the greenhouse to the nursery. The first field planting of yellow-poplar somatic embryo-derived plantlets was made in Spring, 1989. We have also reported on the isolation and culture of protoplasts from yellow-poplar embryogenic suspensions, from which we have been able to regenerate somatic embryos and plantlets of protoplast origin (Merkle and Sommer 1987).

Although the embryogenic cultures which we initiated in 1984 produced thousands of embryos, there remained problem areas which limited the usefulness of the system for large scale production of plantlets. One of these was our ignorance regarding the optimum explant for use in initiating embryogenic cultures. Although we determined that only immature zygotic embryos had the potential to intiate embryogenic callus, we did not know if one particular developmental stage had greater potential than other stages. Neither did we know if the ability to produce embryogenic callus was genotype-specific, since all cultures in our first report originated from a single mother tree. There were also problems at the other end of the process, in the area of conversion of somatic embryos to plantlets. Although thousands of somatic embryos were produced, over 99% were malformed, with fused cotyledons. These malformed embryos, almost without exception, failed to convert. A number of these embryos germinated, if germination is defined as radicle elongation. However, the shoot apices failed to develop. Malformation and low conversion rate were even worse problems when somatic embryos were differentiated in liquid media shake cultures. Until the study described here, we had never obtained a plantlet from an embryo raised in liquid medium.

The research reported here was undertaken with the goal of overcoming the culture initiation and conversion problems described above, with the following objectives: (1) to find the optimal developmental stage of zygotic embryo for explanting to obtain embryogenic cultures, and to look

for biochemical markers to help identify that stage, and (2) to discover methods for the high-frequency production of well-formed somatic embryos with a high conversion percentage.

MATERIALS AND METHODS

Explant optimization

Our approach to determining the optimal zygotic embryo developmental stage for explanting was to sample developing fruit from a number of controlled crosses made at the University of Tennessee's yellow-poplar breeding orchard in 1987 and 1988 by Dr. S.E. Schlarbaum and Mr. R.A. Cox. Fruit were sampled at 2-week intervals throughout the summer and early fall of each year. In 1987, samples were collected at 10, 12, 14, 16 and 18 weeks post-pollination. In 1988, samples were collected at 4, 6, 8, 10, 12, 14, 16 and 18 weeks post-pollination. As each sample was received, the samaras of each cross were divided into 2 subsamples. In subsample 1, samaras were surface-sterilized and dissected, and the embryos and endosperm were placed on solid conditioning medium as described in Merkle and Sommer (1986). Explants and callus growing from them were transferred monthly to fresh conditioning medium and at the end of 3 months, the cultures were scored for production of embryogenic callus. In subsample 2, samaras were dissected and embryos were measured for total length, cotyledon length and hypocotyl thickness. Then, the embryos were placed in 2DMH sample buffer (Mayer et al. 1987) and stored at -70 C until all samples had been received. Stored embryos were assayed for protein developmental markers by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with a Bio-Rad Mini-Gel (8 cm wide by 7 cm long) apparatus, using 7 or 10% polyacrylamide, run at 150 V (constant voltage) for approximately 45 min. Proteins were visualized in the gels either by staining with Coomassie Brilliant Blue R-250 or by silver staining using a protocol supplied by R.P. Feirer (Institute of Paper Chemistry, Appleton WI, personal communication). Buffer, gel and stain recipes used can be found in Sotak (1989). We then tried to relate sampling date, embryo measurements and quantitative and qualitative changes in embryo proteins to the potential of an explant to initiate an embryogenic culture.

Improving embryo form and conversion rates

Our first step in raising the numbers of plantlets we could obtain from each culture was to use embryos which differentiated on solid induction medium. Our standard procedure with our first cultures was to wait until embryos began to green-up and elongate before picking individual well-formed embryos off of the plates and moving them to RW medium to complete conversion. Since the embryos were at least 5 mm long at this point, it meant we were actually waiting for conversion to begin before transferring the embryos. Using this method, a typical yield of 10-20 plantlets per gram of embryogenic callus moved to a plate of induction medium was achieved. Given that some clonal lines produced thousands of embryos per gram of callus, the conversion rate was 1% or less. Our first attempt to improve the conversion rate was to make early selections of well-formed embryos at the 1.5-2 mm torpedo stage, prior to greening. This size corresponds to

that of a mature zygotic embryo, and selection required the use of a dissecting microscope. The primary criterion for selecting an embryo was that it have 2-3 separated cotyledons. Usually 20-30 such embryos could be picked off of a plate every 2 weeks for approximately 2 months. Individual embryos were transferred to one of four "secondary" media (induction medium, induction medium minus CH, induction medium minus CH and solidified with agarose instead of agar, and induction medium minus CH and with only 1% sucrose instead of 4% sucrose) for 7-10 days, scored for the presence of expanded and greening cotyledons, transferred to RW medium and scored for conversion after 1 month.

Additional experiments to improve embryo form and conversion percentage were conducted using embryogenic suspension cultures as the starting point. Suspension cultures were used for a number of reasons. First, suspension cultures have the potential to be "scaled-up" into systems such as bioreactors, where very large numbers of propagules may be produced with small amounts of labor, compared to cultures grown on solid media. Secondly, using suspension cultures as the starting point had the advantage of allowing synchronization of the developing embryos by fractionation of the proembryogenic masses (PEMs) and embryos on sieves. Such a synchronization would also enhance any scale-up for mass propagation since a bioreactor-type system would only be useful if it produced large numbers of mature embryos at the same time. However, our main reason for fractionating the embryos was that we felt that only by observing our treatment effects on synchronized embryos would we be able to determine if our treatments aimed at producing well-formed embryos were effective.

Our fractionation/synchronization procedure was based on that used by Giuliano et al. (1983) with carrot somatic embryos. Briefly, approximately 1 g of PEMs growing in liquid conditioning medium was placed in liquid induction medium. PEMs were sieved on a 140 µm stainless steel screen and the fraction that passed through was sieved once more on a 38 µm screen. The fraction remaining on the 38 µm screen was saved and cultured for one week in induction medium. Then the developing embryos derived from the PEMs were sieved on a 230 µm screen, and the fraction that passed through was sieved on a 140 µm screen. The fraction remaining on this screen was saved and cultured for 1 week, producing embryos that were, at least in gross appearance, synchronized at the early torpedo stage. Then, embryos were moved to solid induction medium for conversion.

Treatments aimed at improving embryo form and conversion percentage were of two types: (1) those imposed during synchronization in liquid induction medium, and (2) those imposed post-synchronization, following transfer of embryos from liquid induction medium. Treatments imposed during synchronization were primarily aimed at producing well-formed embryos, i.e. with 2-3 distinct cotyedons, not fused in a multiple-embryo cluster, and with no premature radicle elongation. We assumed that well-formed embryos would have a high probability of converting. Treatments consisted of the supplementation of liquid induction medium with varying levels of abscisic acid (ABA), mannitol, sorbitol or sucrose. ABA was of particular interest, since Ammirato (1974) showed that ABA at the proper concentration in liquid medium produced well-formed caraway somatic embryos with distinct cotyledons

and unexpanded radicles. ABA levels tested ranged from 10^{-7} M to 10^{-5} M, mannitol and sorbitol each from 4 to 8% and sucrose from 1 to 12%. Postsynchronization treatments were tested for their ability to increase conversion of well-formed embryos. These included rinsing embryos in distilled water, cold (stratification) treatments for up to 9 weeks, desiccation in empty Petri plates, and supplementing the solid induction medium with gibberellic acid (GA), 6BA, or activated charcoal. Following 1 week on solid induction medium (or modified induction medium), embryos were transferred to RW medium to complete conversion and plantlet growth. After 1 month on RW medium, the percentage of embryos successfully converted to plantlets was determined.

RESULTS AND DISCUSSION

Explant optimization

The percentage of cultures that became embryogenic from the 8 crosses made in 1987 showed a maximum of approximately 17% at the earliest (10 weeks postpollination) sampling date and declined to near 0% for the last (18 weeks postpollination) sampling date (Fig. 1). The curve indicates that we probably missed the true maximum of embryogenic potential by not beginning our sampling early enough in the season. Therefore, we began sampling of developing fruit 6 weeks earlier the following season. As a result, the percentage of cultures that became embryogenic from the 6 crosses made in 1988 rose from near 0% for cultures initiated 4 weeks postpollination to a high of approximately 28% for cultures initiated 8 weeks postpollination, and dropped to 0% for cultures initiated from mature embryos 18 weeks postpollination (Fig. 2). Measurements made on zygotic embryos indicated that those with the highest potential to initiate embryogenic cultures were at the globular to early heart stages (0.2 mm or less in diameter), during which they are likely to be undergoing growth by rapid cell division (vs. growth by cell elongation).

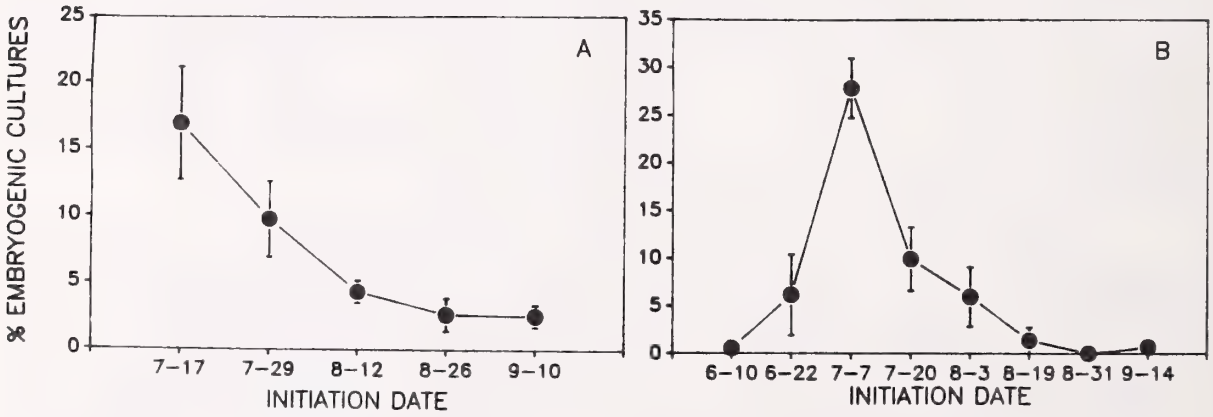

Fig. 1. Percentage of zygotic embryo explants producing embryogenic callus after 2 months on conditioning medium, from 1987 (A) and 1988 (B) Tennessee crosses. Data points represent the means of 8 full-sib families for 1987 and 6 full-sib families for 1988.

Prior to 10 weeks post-pollination, zygotic embryos were too small to isolate for use in protein analysis, so SDS-PAGE could only be used to visualize proteins from embryos from 10, 12, 14, 16 and 18 weeks postpollination. However, since the 10-weeks postpollination embryos showed the second highest embryogenic potential (Fig. 2), we believed comparisons among these stages would still be useful. The most conspicuous marker was a protein of approximately 55 kD, which showed a large increase in abundance from the first through the fifth sampling dates, correlating with an increase in embryo size and a decrease in embryogenic potential. This polypeptide was barely visible 10 weeks postpollination, but became easily visible at 12 weeks and was the most abundant protein for weeks 14-18. A protein with apparent molecular weight of 62 kD was present at 10 and 12 weeks postpollination, but disappeared in embryos from weeks 14-18, when embryogenic potential dropped to essentially 0. The identity of these proteins is unknown, but it is possible that the 55 kD polypeptide is a seed storage protein.

Improving embryo form and conversion rates

Early selection of well-formed somatic embryos at the torpedo stage of development for movement to a secondary medium resulted in an average (over 3 clonal lines and 4 media) of 68% embryos with expanded and greening cotyledons and 37.3% conversion to plantlets one month following transfer to RW medium. Of the secondary media used, induction medium minus CH gave significantly (α = .05) higher percentages of embryos with expanded and greening cotyledons (84.6%) and significantly higher conversion percentages (63.3%) than did regular induction medium (40.9% and 16.2% respectively). Lowering the sucrose concentration to 1% or substituting agarose for agar in the CH-free induction medium did not significantly change the conversion percentage from that of regular CH-free induction medium.

For embryogenic suspension cultures, we found that fractionation on stainless steel screens worked quite well in gross synchronization of developing embryos. However, fractionation did not completely eliminate the production of clusters of multiple embryos from the PEMs. It also failed to raise the frequency of well-formed embryos in liquid media. Embryos continued to enlarge and elongate without developing distinct cotyledons.

The addition of ABA to the liquid induction medium had an observable impact on the development of fractionated somatic embryos. Depending on the clonal line used, ABA at a concentration between 10^{-7} M and 10^{-5} M prevented the production of multiple embryos, inhibited elongation of embryos and raised the frequency of embryos with 2-3 distinct, separated cotyledons. In general, concentrations lower than 10^{-7} M had little apparent effect on somatic embryo development, while concentrations higher than 10^{-5} M completely inhibited development of embryos. In the latter case, embryos would rarely develop past the globular stage and loose cells would proliferate around the periphery of the embryos. For most lines tested, the ABA concentration producing the highest proportion of well-formed embryos (approximately 20-30%) was 5 x 10^{-7} M. When the fractions that did not pass the 140 µm mesh in the first fractionation or the 230 µm mesh in the second fractionation were cultured in the same concentration of ABA, embryo

development was less affected, resulting mainly in the production of larger, malformed embryos. Thus it appears that for ABA to have an effect on yellow-poplar somatic embryo development, the embryos must be exposed to it at an early stage, which we selected for by culturing the smaller fractions.

Mannitol or sorbitol at 8% had similar effects to ABA in that the elongation of embryos was inhibited, but in general, cotyledon development was not enhanced by addition of these osmotica at any concentration we tried. However, raising the sucrose concentration of the induction medium from 4 to 10% had very similar effects to that of ABA in that many embryos formed distinct cotyledons, although we did not quantify the percentage of well-formed embryos for this treatment.

Although both ABA and sucrose appeared to have a positive effect on the production of well-formed embryos, once these embryos were transferred to solid medium, they failed to convert to plantlets at a rate higher than embryos not selected for distinct cotyledons. Thus our assumption that well-formed embryos would convert at a high frequency, based on our experience with embryos differentiated on solid medium, did not apply to embryos differentiated in liquid medium. Furthermore, none of the post-synchronization treatments listed above succeeded in raising the frequency of conversion of liquid-differentiated embryos above the few plantlets obtained by simply transferring embryos to regular induction medium. Of the thousands of well-formed embryos differentiated in liquid medium to date, we have obtained less than 100 total plantlets.

We believe that the long period of time somatic embryos spend differentiating in shake culture may be responsible for their poor performance once transferred to solid medium, since well-formed embryos differentiated on solid medium which appear the same outwardly as liquid-raised embryos have a very high conversion percentage. It is also possible that residual ABA from the liquid medium prevents conversion. Currently, we are attempting to combine the high frequency production of synchronous somatic embryos in liquid medium with the higher conversion rates of embryos differentiated on solid medium by fractionating embryogenic suspension cultures at the PEM stage and immediately plating the selected fractions on solid induction medium. Experiments are underway to determine which modifications of the solid induction medium will give the most efficient plantlet production.

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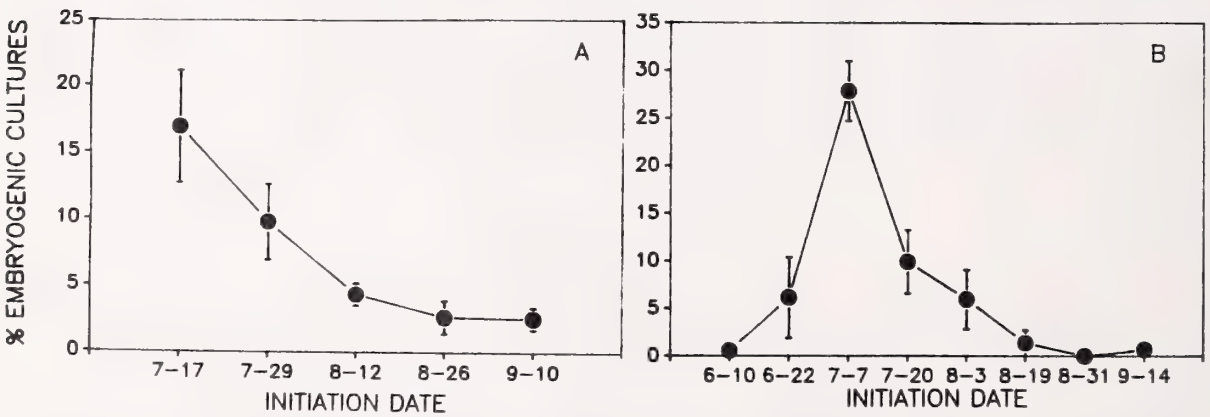

Fig. 1. Percentage of zygotic embryo explants producing embryogenic callus after 2 months on conditioning medium, from 1987 (A) and 1988 (B) Tennessee crosses. Data points represent the means of 8 full-sib families for 1987 and 6 full-sib families for 1988.

ENHANCED GERMINATION OF NORWAY SPRUCE SOMATIC EMBRYOS

M. R. Uddin*, K. Keinonen-Mettala**, and R. J. Dinus*

Abstract.-- Prompt germination and vigorous development of somatic embryos is prerequisite to their efficient utilization in mass clonal propagation of forest trees. A number of factors: e.g., growth regulators, media composition, light versus dark culture, and position of embryos on media, have been shown to affect germination. In experiments evaluating these and other factors, improved germination was obtained by exposing Norway spruce (Picea abies) embryos to darkness for 10 days and then transferring them to light culture conditions. Incubation under low intensity yellow light also enhanced germination, as did laying embryos horizontally on media surfaces. Half-strength DCR medium seemed to give the best germination, although differences among media treatments were small and highly variable.

Keywords: Picea abies, germination, growth regulators, light quality and intensity, somatic embryogenesis

INTRODUCTION

Somatic embryogenesis holds much promise for rapid and inexpensive mass clonal propagation of the best genetically improved forest trees. The process evokes a vision of a revolutionary regeneration system capturing larger genetic gains, greater product uniformity, and more versatility than the traditional seed orchard and nursery approach. The many advantages and recent progress are summarized for conifers by Becwar (in press).

Somatic embryogenesis has been obtained in a number of commercially important conifers, but conversion of embryos to seedlings remains a limiting step. Considerable research has been done on treatments to stimulate germination, especially with Norway spruce (Becwar et al. 1987a, von Arnold and Hakman 1988), but yields of seedlings remain low in proportion to numbers of available embryos. In addition, much labor is required and methods are far from reliable.

Norway spruce somatic embryos produced in our laboratory resemble their zygotic counterparts in terms of most outward characteristics. Even so, we have had variable success in germinating them and securing healthy seedlings in quantity. Factors affecting germination have therefore been the focus of much research in recent times. In this report, we summarize results from a series of five experiments undertaken to promote somatic embryo germination.

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MATERIALS AND METHODS

Norway spruce somatic embryos used in all experiments were obtained from embryogenic callus line, (NS-1)5. This line was initiated from an immature zygotic embryo, has been maintained in our laboratory for a number of years, and has behaved predictably in extensive tissue culture and biochemistry research. Protocols for initiation and maturation are given elsewhere (Becwar et al., 1987b).

To evaluate treatment effects on germination, presumably mature embryos (cotyledonary stage) were removed from callus clumps growing on maturation media and aseptically placed on 25 mL of germination media in 15 X 100 mm plastic Petri plates. Embryos were laid horizontally on media surfaces in all experiments; however, the fifth trial also tested effects of inserting cotyledons in media.

Germination media consisted of either modified half-strength DCR medium (Gupta and Durzan, 1985) or half-stength MS medium (Murashige and Skoog, 1962) without growth regulators. Modified DCR medium differs from the original formulation in that nitrogen sources were altered to include 25 mg/L glutamine and 25 mg/L NH_4NO_3. Media pH's were adjusted to 5.7 prior to autoclaving. Both media were solidified with 0.7 percent agar and supplemented with 3 percent sucrose.

All experiments included cultures grown in a light culture room, intensity = 24 $\mu E/m^2/s$ from cool white florescent and incandescent lights, photoperiod = 16 hr light and 8 hr dark, temperature = 21°C and relative humidity = 70 percent. In trials testing exposure to darkness, cultures were maintained in a totally dark culture room for 10 days (same temperature and humidity as the light room) and then transferred to the light room.

Tests of different light qualities and intensities were performed in the light culture room, with spectra altered by wrapping individual Petri plates in a single layer of yellow, red or blue acetate paper (#12, 26 and 65; Rosco Lux Gel, Reimer Photomaterial Co.). Low intensities were achieved by covering plates with a layer of "Kimwipes" tissue. Incident energies were measured at the top of culture plates with a Licor, Intregrating Quantum Photometer/Radiometer (Model #Li-188B).

Germination was scored 21 or more days after treatments were initiated, depending upon when primary roots were 2 mm long. Root growth (mm) was measured at the end of two experiments. Individual replications (Petri plates) contained 20 embryos, and each experiment included two or more replications. Where applicable, percentages or means per replication were subjected to Analyses of Variance, with differences among means contrasted via Duncan's New Multiple Range Test. All tests of significance were at P = 0.05.

Detailed descriptions of designs and treatments are given in the discussion of individual experiments.

RESULTS AND DISCUSSION

A variety of approaches have been used to stimulate germination of Norway spruce somatic embryos. Media composition has been tested by many workers, light vs. darkness by von Arnold and Hakman (1988), and positioning of embryos on media by Becwar et al. (1987a). Useful leads are also available from other crops. In research on soybean (Glycine max); e.g., workers have used indolebutyric acid (IBA) (Ranch et al. 1986), gibberillic acid (Ghazi et al. 1986), and other growth regulators, either singly or in combination (Lazzeri et al. 1985) as well as desiccation (Hammatt and Davey 1987, Parrott et al. 1988). Furthermore, pulse applications of IBA have been shown to stimulate root initiation on shoots from cultures of Norway spruce cotyledons (Bornman 1983).

Given this background, our first two experiments tested effects of continuous incubation with and 12 hr pulses of varying concentrations of IBA. The experiments were performed on half-strength DCR medium in the light.

Both continuous and pulse application of IBA reduced germination below that observed in the absence of IBA (Tables 1 and 2). Percent germination declined markedly as IBA levels increased. In addition, continuous incubation produced multiple malformed roots at concentrations above 1 mg/L.

Table 1. Germination of Norway spruce somatic embryos after 21 days of continuous incubation with and without IBA*.

IBA (mg/L)	germination (%)
0.0	76.1 a**
0.1	50.5 b
1.0	25.5 c
10.0	18.5 c

* Number of embryos per treatment = 80, 20 in each of 4 replications.
** Means followed by different letters are significantly different, P = 0.05.

Table 2. Germination of Norway spruce somatic embryos at 21 days in response to 12 hr pulses with four IBA concentrations*.

IBA (mg/L)	germination (%)
0	45.0a
1	35.0a
10	21.0b
100	16.0b

* Number of embryos per treatment = 80, 20 in each of 4 replications.
** Means followed by different letters are significantly different, P = 0.05.

Though contrary to expectation, such results are not without merit. Auxins, such as IBA, typically stimulate adventitious root initiation and inhibit subsequent root elongation. Depression of germination by IBA therefore provides indirect evidence that the embryos had developed more or less normally. The considerable variability among experiments (76 versus 45 percent) in the absence of IBA, however, suggests that embryos were not all at the same stage of development or that some treatment is necessary to maximize germination.

Our third experiment tested the effects of incubation on different media or combinations thereof in the light, either continuously or after exposure to darkness for 10 days. Work elsewhere (von Arnold and Hakman 1988) indicated that exposure to darkness for three weeks or less fostered germination, and preliminary trials in our laboratory suggested that 10 days of darkness was an appropriate exposure length.

Percent germination peaked 38 days after the experiment began, and major differencs were noted between embryos exposed to darkness and those maintained in the light (Table 3). Results in the light were similar to those from control treatments in the earlier IBA experiments (Tables 1 and 2), and comparable to the best obtained under similar conditions by Becwar et al. (1987a).

Table 3. Percent germination and root growth of Norway spruce somatic embryos at 38 days as affected by various combinations of media and light/dark culture conditions*.

treatments	germination (%)	root length (mm)
Darkness, 10 days, then Light		
1/2 DCR, Only	82	4.0 ± 2.5**
1/2 MS, Only	94	7.6 ± 4.7
1/2 MS (15 days), then 1/2 DCR	100	6.8 ± 4.1
Light Only		
1/2 DCR, Only	50	3.4 ± 3.2
1/2 MS (15 days), then 1/2 DCR	65	2.6 ± 2.2

* Number of somatic embryos = 40, 20 in each of 2 replications.
** Mean ± stan. dev.

Exposure to darkness substantially increased germination, and effects were consistent across media (Table 3). Though smaller, media effects suggested that half-strength MS medium aided germination.

Darkness also tended to increase root growth, but effects were quite variable. Media composition had lesser effects, suggesting that both media are suitable and that transfer among them is unnecessary.

Although differences were not especially large, exposure to darkness and half-strength MS medium gave the best overall combination of germination and root growth. Moreover, such treatments tended to hasten germination, promote root growth, and produce healthier seedlings.

In general, these findings confirm earlier observations about the merits of exposure to darkness (von Arnold and Hakman 1988), and responses exceeded those to various treatments tested earlier in our laboratory (Unpublished data, The Institute of Paper Chemistry, and Becwar et al. 1987a). That germination peaked at 38 days, however, indicates need for even better treatments or improved maturation protocols to ready embryos for germination.

The beneficial impact of dark exposure led to questions concerning possible effects of differing light qualities and intensities. Our fourth experiment thus evaluated responses to yellow, red, and blue light at two levels of intensity in the light room. Tested also were two levels of white light, and dark exposure for 10 days followed by transfer to the lower level of white light. Embryos were cultured on half-strength MS medium, and germination was scored at 25 days.

Differences among treatments were significant (Table 4), with low yellow light producing the best results. Yellow light and exposure to darkness gave the next best germination, and had approximately equal outcomes. The only other significant difference between intensities within light qualities occurred for the red treatment. Red and blue light produced results more or less equivalent to those observed for white light. Germination both in white light and after exposure to darkness was less than in preceding experiments. This continuedvariability among experiments most likely reflects irregular embryo maturation.

Although not measured, root and shoot growth of embryos given yellow light appeared greater and more uniform than in other treatments. In terms of overall development and vigor, however, seedlings derived from both yellow light treatments and the dark exposure were judged roughly equivalent.

Collective results from our experiments with darkness and light quality or intensity, viewed another way, suggest that Norway spruce somatic embryo germination is inhibited by certain light qualities. This behavior contrasts with that of Norway spruce seed, which germinate readily in light or darkness (U.S.D.A. Forest Service 1974).

The fifth and last experiment was designed to re-examine some of the best treatments from previous trials - culture on half-strength MS medium and exposure to darkness. Also tested were two ways of positioning embryos on media surfaces; laying them horizontally on the surface (Horizontal) and inserting their cotyledons into the media (Inverted). Positioning was included as a factor inthat numerous workers have found that agar inhibits root growth. Inserting cotyledons in media, with embryos either inverted or hanging, was

expected to exploit ability of cotyledons to absorb and convey nutrition to embryos. Earlier work by Becwar et al. (1987a) showed that embryos placed on agar slants or hanging by their cotyledons averaged roughly 50 percent germination, almost twice that of embryos with radicles embedded in the medium. Embryos on agar slants, however, had the best root growth.

Table 4. Germination of Norway spruce somatic embryos as affected by light quality and intensity*.

treatment	germination (%)	irradiance $\mu E/m^2/s$
Darkness for 10 Days, then Low white Light	62.5 c**	5.86
Light Only		
White	23.8 a	9.13
Low white	31.3 a	5.86
Blue	21.3 a	1.71
Low Blue	25.8 a	1.22
Red	36.3 b	3.73
Low Red	25.0 a	2.51
Yellow	66.3 c	4.86
Low Yellow	82.5 d	2.70

* Number of embryos per treatment = 60, 20 in each of 3 replications.
** Means followed by different letters are significantly different, P = 0.05.

Results largely reflected those from preceding experiments, and clearly confirmed the considerable benefits of exposure to darkness (Table 5). Though significant, media effects were smaller than those of darkness, and somewhat at variance with earlier results. That is, half-strength DCR medium proved significantly better than half-strength MS medium. Once again, however, transfer from the former medium to the latter gave no benefit, indicating that this laborious step is unnecessary.

Embryo positioning effects were also significant, with Horizontal embryos having roughly 10 percentage points greater germination than Inverted ones. Such findings conflict with those of Becwar et al. (1987a) who reported that embryos with cotyledons embedded in medium germinated best. In their experiment, however, embryos were hanging rather than inverted as in the present experiment. Exposure to darkness thus seems the factor of greatest importance, with media composition and embryo position having beneficial but lesser effects.

Table 5. Germination of Norway spruce somatic embryos at 21 days as influenced by various combinations of media, light/dark culture conditions, and embryo position on media surfaces*.

treatments	germination (%) inverted	horizontal	mean
Darkness, 10 days, then Light			
1/2 DCR, Only	86.3a **	93.8a	90.0a
1/2 MS, Only	76.3a	67.8b	72.0b
1/2 MS (15 days), then 1/2 DCR	48.8b	68.0b	58.4c
Light Only			
1/2 DCR, Only	43.8b	68.8b	56.3c
1/2 MS, Only	30.0c	35.0c	32.5d
Mean	57.0	66.7	61.8

* Number of embryos per treatment = 80, 20 in each of 4 replictions.
** Means within columns followed by different letters are significantly different, P = 0.05.

CONCLUSIONS

Results of the present work indicate that germination of Norway spruce somatic embryos can be stimulated and enhanced via manipulation of media composition, dark versus light culture conditions, and positioning of embryos on germination media. While such manipulations apparently provide appropriate stimuli, continued variability among experiments, relatively low germination percentages, and long periods of time to maximum germination all suggest that embryos may not have developed uniformly, or that they are not sufficiently mature to respond to the stimuli.

Somatic embryos of Norway spruce and other conifers produced in our laboratory appear to develop normally, and to recapitulate most stages in zygotic embryogenesis. Though smaller, they closely resemble their zygotic counterparts in terms of general morphology and anatomy. Some important differences, however, are apparent, and these may bear on germination and conversion to seedlings. Given current methodology, somatic embryos typically accumulate lesser quantities of storage compounds than zygotic embryos (Feirer et al. 1989), and lack access to these and other compounds from female gametophytes. In addition, they do not experience changes that occur in and around zygotic embryos during the final phases of seed ripening and drying.

In sum, our manipulations apparently provided needed stimuli, but did not substitute effectively for complete maturity, accumulated reserves, or other factors governing readiness for germination. Possible avenues to ensure maturity and readiness include incubation with abscisic acid during the maturation phase (Becwar et al. 1987a), and dessication before germination to simulate natural seed ripening and drying. Research on these and other approaches is underway.

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GENETIC ENGINEERING AND TRADITIONAL BREEDING: WHERE IS THE INTERFACE?

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Abstract. -- Propagation of genetically superior stock a cornerstone of any genetic improvement program. For clonal forestry and genetic engineering to be useful to a tree improvement program, highly efficient methods of vegetative propagation need to be developed. It is well established in crop plants that genotype is an important determining factor in the develop of successful propagation methods. Preliminary data from tree species shows that similar genotypic effects exist in these species. To maximally exploit superior genotypes in ongoing sexual breeding programs, responsiveness to vegetative propagation needs to be selected to insure continuing flow of genotypes among sexual breeding, clonal forestry and genetic engineering efforts. Future research should be undertaken to determine the nature and degree of heritability of vegetative propagation in valuable tree species. In addition a highly regenerable select nuclear population should be created as the common pool through which genetically engineered genotypes could be moved into clonal forestry or sexual breeding programs.

Keywords:Clonal forestry, genetic engineering, sexual breeding, genotypic effects, vegetative propagation

INTRODUCTION

One of the cornerstones upon which any crop improvement program rests is the ability to move individuals with improved genotypes into operational field plantings. There are two methods for producing commercial quantities of selected genotypes, either through seedlings or via some form of vegetative propagation. The ideal propagation approach should allow exploitation of both the additive genetic gain achieved through sexual breeding and the non-additive gain identified during testing for superior phenotypes. In addition, the propagation method should produce plants at very low costs and in the large numbers required for commercial cropping. It is also important that the propagation method be highly efficient for all selected

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genotypes, otherwise valuable genotypes will be lost to commercial growers. Finally, with the advent of biotechnology, it is important that the propagation method interface with both sexual breeding programs and gene transfer methods employed with genetic engineering approaches to crop improvement.

In forest tree improvement programs, the traditional method of propagating improved genotypes for operational plantings is through seedlings. The advantages of this method are that the additive genetic gain is captured, and production from mature trees of tested superiority is highly efficient and inexpensive. Although there is variability among families, all genotypes within the select breeding population can be exploited at reasonable cost. However, sexual breeding does not allow capture of the non-additive genetic gain identified during progeny testing. Also, the long breeding times for tree species greatly slows the rate of genetic improvement. Another disadvantage is that the sexual breeding process can interface with genetic engineering only indirectly, after a select genotype has been produced using vegetative propagation methods.

Asexual, vegetative propagation methods have been explored for their usefulness with forest tree species. These methods include rooted cuttings, micropropagation (the induced growth of pre-existing, undeveloped buds), and various tissue culture methods (including adventitious shoot formation and somatic embryo formation). These methods have the advantage of capturing both the additive and non-additive genetic gain of select individuals. Also these methods can be used to propagate superior individuals produced via sexual breeding and are used to produce transgenic plants (plants carrying a newly inserted gene) created through genetic engineering. However, vegetative propagation methods have a number of disadvantages. Propagules, even those produced by rooted cuttings, are more expensive than seedlings. Vegetative propagation methods lose much of their efficiency when the source of material is from old, mature trees. In addition, an increasing number of studies with a wide variety of plants has shown that vegetative propagation efficiency is influenced by genotype. The forester's ability to move select genotypes to the field using vegetative propagation will be determined by the degree of genetic correlation of tissue culturability with economic traits of interest.

For clonal forestry to become reality and to be usefully integrated into long-term genetic improvement programs, vegetative propagation methods must be developed which can multiply all select genotypes inexpensively and

with high efficiency. Therefore, it is timely to ask if the genotypic dependence of vegetative propagation will present a major obstacle to the development and long-term potential of clonal forestry.

THE NATURE OF THE CORRELATION

Crop Plants

The existence of genotypic effects on vegetative propagation is well documented in crop plants including common bean, rice, tobacco, tomato, sweet clover, alfalfa, potato, petunia, corn, wheat (Keyes et al., 1980, and literature cited therein) and sweet potato (Templeton-Somers and Collins, 1986). Genotypic effects have been observed using a variety of culture methods, including callus growth and shoot regeneration (Templeton-Somers and Collins, 1986; Mok and Mok, 1977), root formation (Keyes et al., 1980), anther culture (Phillips and Collins, 1977), and somatic embryogenesis (Chen et al., 1987; Brown and Atanassov, 1985). Observation in red clover (Keyes et al., 1980), alfalfa (Chen et al, 1987), corn (Tabata and Motoyoshi, 1965), and sweet potato (Templeton-Somers and Collins, 1986) showed statistically significant additive genetic effects for both tissue growth and differentiation. Such heritable variability indicates that the frequency of genotypes more responsive to tissue culture techniques can be raised by genetic selection.

Tree Species

The existence of a correlation between genotype and vegetative propagation has also been observed for tree species. Zobel et al (1983) have reported 64-92% rooting ability for cuttings from 25 select Eucalyptus clones at Aracruz, Brazil. Frampton (personal communication) has observed variation in rooting ability of loblolly pine cuttings. Similar variation has been seen with sweetgum (Cunningham, personal communication) and with Eucalyptus species in commercial propagation nurseries in South American (Kellison, personal communication). Variation at the species, family, and individual plant levels has been observed during the development of tissue culture methods as well.

At the species level, Bergmann (this meeting) reports that direct extrapolation of the highly efficient method developed for shoot regeneration in Pinus radiata does not

work for several pine species, although good response was seen with Pinus eldarica. This is not surprising, as it is generally accepted that tissue culture methods need to be independently developed for each plant species of interest.

At the family level, Amerson (personal communication) has observed that shoot regeneration from loblolly pine cotyledons cultured on cytokinin-containing medium shows considerable family and seed-to-seed variation. Similar observations have been made by Aitken-Christie (personal communication) in Pinus radiata. The Forestry Research Institute in New Zealand is currently working on methods to mechanize meristem culture to produce large numbers of plants at prices competitive with seedlings (Aitken-Christie et al, 1988). Genotypic variation already presents an obstacle to these efforts as only 40% of select genotypes will give meristematic cultures capable of the long-term culture necessary for mechanization (Aitken-Christie, personal communication). Similar family and seed-to-seed variation has been observed in research aimed at the development of somatic embryo culture in loblolly pine (Amerson, personal communication). Glock and Gregorius (1986) undertook a study specifically designed to determine if genotype or culture conditions more strongly influenced growth of birch callus in vitro. Their results showed that the variation in growth was more strongly determined by genotype than culturing environment.

The existence of genotypic effects on vegetative propagation is well documented, however the nature of the correlation is not. If a positive correlation exists between the ability to vegetatively propagate and traits of economic importance, positive selection for vegetative propagation is already occuring. Aitken-Christie (personal communication) has observed that several of the select families of P. radiata show high efficiency when tissue cultured. However, in vitro work with 23 select families of P. taeda showed shoot regeneration ranging between 10-60 shoots/embryo, with an average of 27.8 shoots/embryo (Amerson, personal communication). Rooting of tissue cultured shoots produced from the same families showed a similar variation. This data is consistent with the hypothesis that the ability to vegetatively propagate does not correlate with currently selected characteristics, such as tree form.

Data from physiological and tissue experiments suggests that the correlation between responsiveness to vegetative propagation and tree form may be negative. It has been observed in many species of plants that cultivars which have strong apical dominance and which do not sucker

readily are often difficult to vegetatively propagate. This observation is so wide-spread that it has become a "rule-of-thumb" to nurserymen and tissue culturists. Yet, strong apical dominance is a highly desirable phenotype in commercial forestry. This raises the possibility that selection for strong apical dominance may, inadvertantly, select for poor responsiveness to vegetative propagation methods.

IMPLICATIONS FOR CLONAL FORESTRY AND GENETIC ENGINEERING

If clonal forestry is to be a useful tool in tree improvement programs, inexpensive mass propagation methods for all select genotypes need to be developed. Sexual breeding programs are continually creating new superior genotypes. If these individuals show random variation in their responsiveness to vegetative propagation methods, clonal propagation will continue to be sub-optimal, unless methods are continually fine-tuned for each new select genotype. The necessity to modify tissue culture methods for highest efficiency for each select genotype will greatly increase costs making it highly unlikely that vegetative propagules will be cost-competitive with seedlings. If a negative genetic correlation exists between desirable tree form and responsiveness to vegetative propagation methods, breeding for form will be breeding towards a technological bottleneck. The best trees will be increasingly difficult to propagate vegetatively. In the long-term, this could preclude the use of genetic engineering for modification of select genotypes from sexual breeding programs.

In addition, gene transfer methods currently in use and those envisaged for the foreseeable future, require tissue culture methods for creating transgenic plants expressing newly inserted genes. Ideally, a genetic engineering approach to tree improvement would utilize tissue from superior individuals in an ongoing breeding program. The efficiency at which these genotypes regenerate plants will determine, to a large extent, the expense of creating transgenic plants. These select transgenic plants must then be multiplied for use either as new individuals to be integrated into an ongoing breeding program, or as cloning stock for mass vegetative propagation for operational plantings. Both of these uses requires efficient tissue culture regeneration.

For sexual breeding and genetic engineering approaches to complement each other, they need to have efficient, technological access to the same base population of genetically select trees. The development of highly

efficient, regenerable lines within existing tree breeding populations would enhance the usefulness of *in vitro* methods for clonal forestry and genetic engineering. Genetic studies should be undertaken to assess the nature and degree of genotypic effects on vegetative propagation. Exisiting select breeding populations could serve as the base for a nuclear breeding program whose goal is to create a population of trees with economically superior characteristics and high efficiencies of vegetative propagation. This population could serve as the common pool through which genetically engineered individuals could be moved into clonal forestry programs or into sexual breeding programs, and the best genotypes could be made accessible to improvement efforts through genetic engineering.

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CONCURRENT SESSION: 3A

EIGHT YEAR RESULTS OF SLASH AND LOBLOLLY PINE PROVENANCE TESTS IN CHINA

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ABSTRACT.--Seeds of loblolly and slash pine were obtained from natural range of the species in the US (loblolly pine, 10 states; slash pine, 6 states). Provenance tests were carried out in 7 locations (latitude range from 21.9-31.8 N) in China in 1981. There was significant variation among different seed sources of loblolly pine in 8-year height, diameter and volume growth. Significant positive correlations were found between growth and mean annual temperature, mean minimum temperature of January, rainfall of June-September, and frost free season; a significant negative correlation was found between growth and latitude. The interaction of seed sources by locations were highly significant for height and volume growth. The best seed sources in southern and central subtropical regions of China were from the coastal regions of Florida, South Carolina, and Louisiana (Livingston). For the northern subtropical region of China, the piedmont or inland seed sources were better than the coastal seed sources due to greater cold tolerance.

The growth pattern of slash pine showed random variation. The correlation of growth with climatic factors of seed sources was not significant. There was also no significant seed source x location interaction. Superior seed sources of slash pine were from Florida, South Carolina and Georgia.

Species comparisons of land races of slash, loblolly and local Masson pine were also included in the tests. In southern subtropical regions of China, Masson pine and loblolly pine showed better performance in high elevations (300-500 m), but slash pine was better at low hill and coastal regions. Loblolly pine and slash pine grew faster than Masson pine in low hill and mountains of central subtropical regions. Loblolly pine was the best in low elevations of northern subtropical regions when compared with local Masson pine and slash pine.

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DROUGHT TOLERANCE IN SIX SWEETGUM CLONES

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Abstract.--Sixteen ramets each of three good and three poor sweetgum phenotypes were exposed to two watering levels for four weeks in a greenhouse. Response parameters focused on stomatal conductance and biomass partitioning to explain possible mechanisms of drought tolerance. Water stress caused significant declines in stomatal conductance. Clones showed little interaction with watering treatment for conductance but exhibited considerable variation within treatments. Analysis of allometric growth indicated an increase in root dry matter at the expense of leaf dry matter in stressed trees. Clones generally did not vary significantly in their relative partitioning of biomass. Clonal responses were independent of ortet field performance.

Keywords: Liquidambar styraciflua L., water stress, stomatal conductance, allometric growth, biomass partitioning.

INTRODUCTION

Site adaptability, good sprouting ability, and relatively high productivity should propel sweetgum (Liquidambar styraciflua L.) into a more prominent position in the southeast United States, particularly for fiber and fuel production in short-rotation, intensive plantations. However, drought can limit tree growth (Bassett 1964, Kramer 1983), especially on well-drained sites. Clones exhibiting a genetically controlled mechanism to tolerate drought should perform better under water stress. Identification of characteristics that impart drought tolerance could allow early screening of genotypes for use on drought-prone sites, thus increasing yields (Kellison et al. 1979, Hennessey et al. 1988).

Several response mechanisms enable trees to tolerate dehydration and maintain a suitable water balance for continued growth. Stomatal regulation allows plants to conserve water at a cost of reduced photosynthesis. Pezeshki and Chambers (1986) found sweetgum to have relatively high stomatal sensitivity, allowing partial stomatal closure at relatively high water potentials to reduce desiccation while affording some positive photosynthesis

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during prolonged drought. Furthermore, trees which partition a greater proportion of carbohydrate to root growth are more capable of delaying dehydration by increasing absorptive area and the volume of soil explored for available water. Kitchens (1985) found that Piedmont sweetgum possesses drought tolerant characteristics such as decreased shoot-root ratios and lower stomatal conductances as compared to coastal sweetgum.

Little work has been conducted to characterize the intraspecific variation in drought tolerance for sweetgum. Therefore, the objectives of this study were to: 1) evaluate the impact of water stress on leaf conductance and biomass partitioning by clone, 2) quantitatively compare clonal variation with respect to watering treatment, and 3) establish whether drought response of young rooted cuttings might explain observed differences in ortet field performance.

METHODS

Study Design

A vegetative propagation procedure similar to that for Eucalyptus (Zobel et al. 1983) was used to root stump-sprout cuttings. Mother trees on a moderately-well to poorly drained site in southeast Virginia were selected for 14-year height by identifying the tallest and shortest individuals in the tallest and shortest three families, respectively. Sprouts were collected and transported to a greenhouse in Raleigh, North Carolina in June 1988. Fifteen-cm cuttings were dipped into a 0.3% IBA solution, stuck in flats of vermiculite:perlite (1:1 by volume), and placed under an intermittent mist system. After seven weeks, cuttings with the greatest root development from each clone were transplanted individually to tall, plastic pots (2.7 liter) containing sand. Mist was gradually reduced over a three-week period and nutrient solution was applied daily. One week prior to treatment initiation the most uniform 16 ramets of each clone were placed on the greenhouse experimental apparatus to acclimate to the well-watered regime. A single row of buffer trees reduced edge effects.

A split-plot design with two watering treatments as main plots and six clones as subplots was replicated eight times (i.e. 96 study trees). Each clone was represented once and arranged randomly within each treatment-replication combination. Clones were subdivided by ortet field performance as good: 310, 316, 338, and poor: 312, 340, 353.

Watering Treatments and Environmental Conditions

Based on a preliminary study, non-stress and stress watering treatments received nutrient solution four times and one time daily, respectively. Deionized water was applied once weekly to avoid salt accumulation. A drip-irrigation system delivered the treatments at approximately 300 ml increments which allowed equal soil wetting because drip-point was exceeded. Watering treatments were initiated in October. Supplemental incandescent lighting (100 Watt bulbs) extended the natural photoperiod to 18 hours. Greenhouse temperature ranged from 18 to 30 C, and relative humidity varied between 10 and 95%.

Measurements

Using a Licor model LI-1600, leaf resistances were obtained at midday nine times during the study, measuring the fourth leaf from the apex on all trees. Initial and final height of the largest shoot was measured from bud origin to the uppermost node. Trees were harvested after four weeks to obtain oven-dry (70 C, 60 h) weights of leaves, stems, and roots.

Statistical Analysis

Analysis of variance was used to test effects of treatment, clone, ortet field performance, and interactions of treatment x clone and treatment x performance on leaf conductance (inverse of resistance), initial and final height, and total dry weight. Duncan's multiple range test was used to detect significant differences among clone means.

Relative partitioning of biomass among plant organs has been shown to change with increasing plant size (Ledig and Perry 1965). Regression parameters were compared to detect differences in dry matter allocation to leaves, stems, and roots using the allometric equation (Bongarten and Teskey 1987):

$$\log (O) = a + b \log (W)$$

where O is the organ dry weight, W is the total plant dry weight, and a and b are constants. Covariance analysis was used to adjust organ dry weight for plant total dry weight and to test significance for watering treatment, clone, and ortet performance differences in regression intercepts and slopes (Freund and Littell 1981, Bongarten and Teskey 1987). A significant difference among regression intercepts indicates a difference in the adjusted treatment or clone means if slopes are similar. A significant difference among regression slopes indicates a change or difference in the relative allocation of dry matter to the plant organ. Where heterogeneity of regression slopes for clones within treatment was significant, pairwise comparisons using the studentized range statistic were performed (Zar 1984).

RESULTS AND DISCUSSION

Stomatal Conductance

For each of the nine measurement days during the study, non-stress trees showed greater stomatal conductance (cm/s) than stressed trees ($p<0.025$) (Figure 1). For both treatments the gradual decline in conductance may have been attributed to increasing stress with increasing plant size. Because both treatments exhibited very similar reductions, the decrease is more likely to have resulted from changing environmental conditions (e.g. light, temperature) caused by lateness in the growing season.

Conductance response patterns among clones were similar within each watering regime, but mean values differed considerably (Figure 2). Clone 340 showed consistently greater conductance than the highly uniform response of the other five clones within the non-stress treatment ($p<0.05$). Conductance for clone 312 decreased sharply relative to the other clones. Clone 353 maintained consistently low relative conductance. Clones of good ortet per-

formance (310, 316, and 338) showed intermediate conductances. Separation among clonal conductances was greater within the stress treatment (Figure 2), but individual clonal response remained consistent with those just described except for clone 316 which showed a large reduction in conductance with stress.

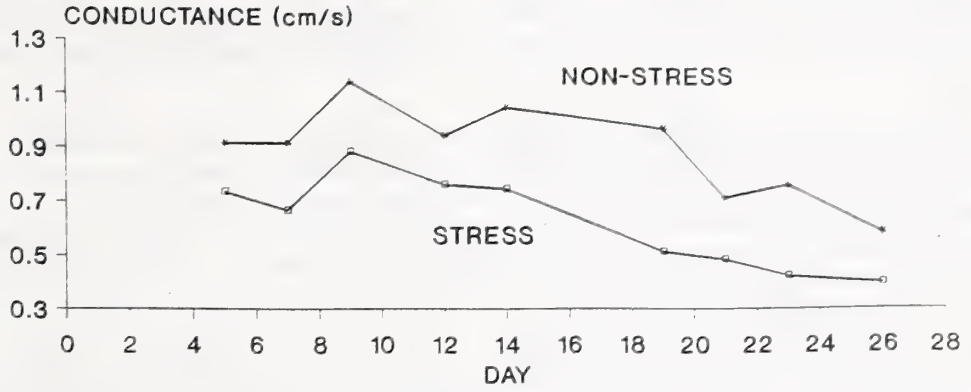

Figure 1. Stomatal conductance as affected by watering treatment across 6 L. styraciflua clones (n=48).

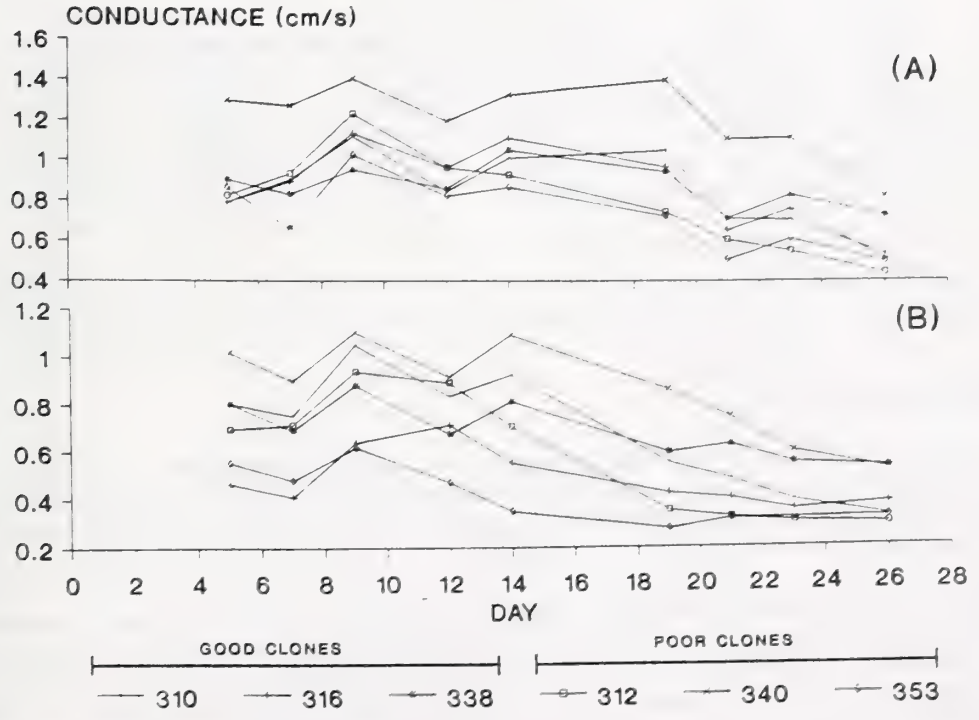

Figure 2. Stomatal conductance for 6 L. styraciflua clones (n=8) as affected by watering treatment: (A) non-stress and (B) stress.

The interaction of clone x treatment was not significant for stomatal conductance at any measurement date. Separation of the interactive effect of plant size x treatment from the clone x treatment effect was difficult due to initial size differences among clones (Table 1). Because larger plants tend to possess greater leaf area, transpiratory losses and degree of water stress will be greater when compared to smaller trees with less leaf area. However, clones 340 and 312 were of comparable initial size (Table 1), but their conductance responses were different (Figure 2). Because it was able to maintain the highest conductance (Figure 2) and total dry weight production (Table 1) relative to watering treatment, clone 340 reflected a high degree of drought tolerance compared to other clones tested (Kelliher and Tauer 1986).

The effect of ortet field performance and performance x treatment interaction were not significant for stomatal conductance at any measurement date. Conductance for poor performers was higher than good performers early in the study, but the values converged within two weeks (Ehlert, unpublished data). Conductances among non-stress good-performers were not significantly different for most measurement days.

Table 1. Initial height, final height, and total dry weight by L. styraciflua clone as affected by watering treatment (n=16 for initial height; n=8 for final height and dry weight). Values within a column followed by the same letter are not significantly different at $p<0.05$.

Clone	Initial Ht.	Final Ht.		Total Dry Wt.	
	(cm)	(cm)		(g)	
		Non-stress	Stress	Non-stress	Stress
Good: 310	4.63 b	32.54 a	23.44 a	6.04 b	5.03 b
316	9.92 a	39.78 a	21.88 a	9.61 a	6.53 ab
338	1.86 bc	31.55 a	18.24 a	6.39 b	5.22 b
Poor: 312	5.12 b	33.05 a	23.36 a	7.88 ab	5.49 ab
340	5.21 b	31.66 a	19.79 a	10.13 a	7.75 a
353	0.74 c	15.41 b	5.31 b	3.14 c	2.21 c
Combined	4.58	30.66	18.95	7.20	5.44

Biomass Partitioning

All clones except 310 exhibited greater total dry weight when well-watered as compared to water-stressed (maximum $p<0.07$). Combined clonal mean for total dry weight was greater for non-stress as compared to the stress treatment ($p<0.01$) (Table 1).

Watering treatment affected biomass partitioning among plant components. Allometric regression parameters for leaves and roots were significantly altered by watering treatment (Table 2). Treatment differences across clones were detected for the intercepts of leaves and roots ($p<0.001$) with no effect on slope (Table 2). Regression parameters for stems were not different by treatment. Thus, for a given total weight, water-stressed cuttings generally had lower leaf weights and greater root weights than well-watered plants.

Table 2. Significance levels for analysis of watering treatment differences in allometric growth of leaves, stems, and roots of 6 L. styraciflua clones. (* indicates $p<0.05$).

Clone	Leaves		Stems		Roots	
	intercept	slope	intercept	slope	intercept	slope
Good: 310	.379	.442	.966	.363	.174	.032 *
316	.003 *	.866	.404	.980	<.001 *	.639
338	.472	.580	.229	.754	.148	.914
Poor: 312	.010 *	.808	.198	.045 *	.039 *	.041 *
340	.112	.262	.157	.585	.004 *	.362
353	.013 *	.405	.116	.439	.061	.601
Combined	<.001 *	.149	.428	.408	<.001 *	.847

Watering treatment effect on the allometric parameters of individual clones deviated from general trends observed for all clones (Table 2). Three clones showed significant treatment differences among leaf intercepts (316, 312, and 353), and four clones had differing root intercepts (316, 312, 340, and 353). Clonal regression parameters were not different between treatments for stems. No treatment differences were detected for any regression parameter of clone 310 or 338 (Table 2). Clone 340 responded uniquely, exhibiting no treatment effect on leaf biomass but a significant increase in stress treatment root weight, imparting further evidence of drought tolerance (Table 2).

Similar allometric response patterns of increased root weight at the expense of leaf weight were found under low nitrogen treatment for sweetgum seedlings and rooted cuttings by Cunningham (unpublished data) and for loblolly pine seedlings by Li (1989). However, loblolly pine seedlings exposed to dry soil conditions allocated proportionately more biomass to roots at the expense of stems rather than needles (Bongarten and Teskey 1987).

Significant clonal effects were observed for the allometric parameters within watering treatment. Differences were observed among intercepts of leaves and stems of well-watered clones ($p<0.02$) (Table 3), which indicated variation in biomass partitioning to leaves and stems (Table 4). Analysis of heterogeneity of individual regression slopes for leaves and stems indicated that all slopes were highly similar except for those of clone 353. As total dry weight increased, clone 353 allocated proportionately less biomass to leaves and more to stems relative to other clones (Ehlert, unpublished data). This response may seem attractive, but growth for clone 353 was significantly lower than other clones (Table 1). Clone 312 exhibited the largest leaf weight and smallest stem weight compared to the other clones when well-watered (Table 4), thus providing a large transpirational surface and perhaps explaining its rapid decline in stomatal conductance (Figure 2).

Clonal effects were detected for intercepts of leaf, stem, and root within the stress treatment, but slopes did not differ (Table 3). Therefore, the relative distribution of dry matter to each component was similar among

clones, but the amounts allocated varied (Table 4). A preferential partitioning of biomass to roots was an apparent response to the stress treatment (Table 4). Compared to other clones, 316 exhibited a particularly greater root biomass production to compensate for the increased water deficits (Table 4).

Partitioning of biomass among clones was independent of ortet field performance.

Table 3. Significance levels for analysis of clonal differences in allometric growth of leaves, stems, and roots within watering treatment. (* indicates $p<0.05$).

	Non-stress			Stress		
	leaves	stems	roots	leaves	stems	roots
intercept	<.001 *	.019 *	.443	.002 *	.051	.017 *
slope	.035 *	.013 *	.097	.942	.351	.221

Table 4. Adjusted (least square) mean dry weight (g) among leaves, stems, and roots within watering treatment for 6 L. styraciflua clones. Values within a column followed by the same letter are not significantly different at $p<0.05$. (# indicates that the value should not be compared to others because of its significantly different regression slope).

Clone		Non-stress Treatment			Stress Treatment		
		leaves	stems	roots	leaves	stems	roots
Good:	310	2.90 b	2.16 b	1.21 a	2.01 b	1.63 a	1.11 b
	316	3.22 ab	1.89 bc	1.21 a	1.90 b	1.34 ab	1.55 a
	338	3.36 a	1.79 c	1.18 a	2.49 a	1.17 b	1.08 b
Poor:	312	3.52 a	1.61 c	1.18 a	2.23 ab	1.26 b	1.26 ab
	340	3.29 ab	1.95 bc	1.08 a	2.15 ab	1.47 ab	1.15 ab
	353	2.16 c #	3.38 a #	1.19 a	1.88 b	1.70 a	0.97 b

CONCLUSIONS

When water stressed the six sweetgum clones exhibited reduced stomatal conductance and increased dry matter allocation to roots compared to leaves. This indicated the influence of available soil moisture on tree growth and physiology. Clonal variation in conductance and dry weight partitioning was evident and suggested intraspecific differences for drought tolerance. The characteristics examined did not explain observed differences in ortet field performance. The artificial study conditions, young plant material, and small number of genotypes qualify these results. Examination of a larger number of clones, particularly from families proven to be fast growers and under field conditions could prove successful in identifying clones with desirable drought tolerance characteristics.

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FIRST-ORDER LATERAL ROOT DEVELOPMENT: SOMETHING TO CONSIDER IN MOTHER TREE AND PROGENY ASSESSMENT

P. P. Kormanik and J. L. Ruehle[1]

Abstract.--Recent work on frequency distribution and heritability of first-order lateral roots (FOLR) of loblolly pine and sweetgum seedlings suggests the desirability of using specific root characteristics of seedlings as a supplement to assessment of stem dimensions. For 3 years we have tested progeny from approximately 75 mother trees in Georgia Forestry Commission seed orchards. Frequency distribution of progeny by FOLR number appears to be highly heritable. Seedling height and root-collar diameter are readily altered by certain cultural practices such as fertilization, top clipping, and root pruning. But, within a standard seedbed density range, numbers of FOLR were affected little by changing cultural practices. At lifting, seedlings could be found with good stem characteristics and poor FOLR development. However, regardless of nursery conditions, few seedlings with excellent FOLR development had poor aboveground development.

Results suggest that it may be possible to select superior mother trees by rating number of FOLR on their progeny produced under specific nursery conditions. Less than 20% of the progeny of some trees have ≤ 3 FOLR; more than 60% of the progeny of others have ≤ 3 FOLR. If results of ratings of these progeny in current field trials coincide with our nursery ratings, we will likely have an additional tool to identify the best mother trees in pine seed orchards.

Keywords: Root morphology, tree improvement.

INTRODUCTION

The Tree Improvement Programs (TIP) in the United States are perhaps the most important innovations in forestry in this half century. In the Southern United States, tree improvement efforts have been concentrated on southern pine species. Extensive seed orchards of selected mother trees and highly mechanized nurseries are integral parts of advanced plantation technology that the TIP made possible.

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One of the major benefits expected from TIP was greater uniformity and higher quality of planting stock. Early research showed that large seedlings tended to perform better than small ones after outplanting and one improvement objective was to increase the percentage of large seedlings in nurseries (Wakeley 1954). Since the mid-1960s, improvement in nursery practices has resulted in marked increases in average seedling size. However, many of the seedlings produced were too large for conventional planting operations, and uniformity was often lost because faster growing seedlings interfered with the development of the slower growing ones. Eventually, some degree of seedling uniformity was restored by mowing seedling tops several times during the latter part of the growing season (USDA Forest Service 1985).

Producing a high percentage of seedlings within specific size ranges became synonymous with improved seedling quality. If seedlings were uniform in size, they were plantable. Now, after 25 years, we recognize that early expectations from improvement in seedling quality are not being realized. To obtain loblolly pine stands of desired stocking, foresters routinely overplant by 40% because experience has shown that a significant proportion of the planted seedlings are usually not competitive. Unfortunately, survival patterns cannot be predicted, so plantation are often overstocked in some places and understocked in others. The conclusion stated by the Southern Industrial Forestry Council (1984) appears valid: "the failure to produce and to recognize seedlings with consistently high establishment and growth potential are major obstacles to successful plantations in the reforestation programs in the southern United States".

Current research at the Institute for Mycorrhizal Research and Development (IMRD) project at the USDA Forest Service Forestry Sciences Laboratory, Athens, Georgia, shows that a significant proportion of the progeny tested in nursery beds, even among phenotypically selected superior mother trees, develop few first-order lateral roots (FOLR). We have found that for either single mother tree progeny or seedlings from mixed seedlots of commercial origin, seedlings with ≤ 3 FOLR are less competitive in the nursery than those with greater numbers of FOLR.

In this paper we describe some of our research on FOLR frequency distributions of 1-0 bare-root loblolly pine seedlings.

Seedling Production

For the past 4 years loblolly seedlings were tested at the IMRD experimental nursery located near Athens and maintained in cooperation with the School of Forest Resources, University of Georgia, Athens. Seedbed density was maintained at approximately $275/m^2$ regardless of fertility practices. The seeds were collected from individual mother trees in the Georgia Forestry Commission's Arrowhead and Baldwin seed orchards. The seedlings were grown in concrete block nursery beds (1.22 m x 18.29 m) with a 25 cm gravel base covered by a 6 mil black polyethylene barrier. Above the barrier was 50 cm of a soil mixture (2:1:1) of loamy forest topsoil, sand, and finely ground pine bark.

Since soil fertility varied from year to year, available soil P (Bray II) and K were adjusted each year to 50 to 75 ppm P and 80 to 100 ppm K. Calcium was maintained in the range of 390-500 kg/ha. Nitrogen was applied in 3 to 5 small applications per year totaling 60 to 160 kg/ha with the final application by mid-July. During germination and initial seedling growth beds were watered frequently as needed, but once seedlings were well established they received 2.5 to 3.0 cm of water per week as rain or supplemental watering.

Seedlings were evaluated in mid-January, and diseased or mechanically damaged seedlings were discarded (usually only 1% of the seedlings). In most tests data were taken on the first 100 seedlings lifted from each family per replicate. Height, root collar diameter (RCD), and number of FOLR were recorded for each seedling and a subsample of eight seedlings per replicate was obtained for destructive sampling. The subsample was divided into four FOLR categories (0-3, 4-5, 6-7, and $\geq$8) that permitted these data to be approximately proportional to the population. Seedlings of mixed seedlot origin provided by two to four state or industrially operated nurseries were also tested and their FOLR frequency distributions compared closely to the theoretical one reported in Kormanik and Muse (1986).

RESULTS AND DISCUSSION

Experimental Nursery Results

The yearly combined FOLR frequency distributions of the progeny from all family seedlots tested during these 4 years compared favorably with the theoretical distribution developed and reported earlier (Kormanik and Muse 1986). In all of the more than 70 families of progeny that were tested, the relative competitive position of seedling in the nursery was correlated with FOLR numbers; in each sibling group, the smallest seedlings had the fewest FOLR. Family mean heritability estimates for FOLR numbers have been uniformly high in all tests. The average family mean heritability estimates have been 0.76$\pm$0.11 for the different families tested (Kormanik, unpublished data).

While the combined FOLR frequency distribution compared favorably to the theoretical distribution, the individual family distribution did not. Some families produced a high percentage of "carrot-rooted" individuals while other families produced a low percentage. This variation was quite evident in the quality and uniformity of the seedlings within specific half-sib progeny groups. The spread between a poor and a good family, based on FOLR frequency distribution is shown in Figure 1. The better families have fewer than 25% of their progeny with $\leq$3 FOLR, but the poorer families have about 60% of their progeny in this group. Within all families tested, seedlings in the 0-3 category have always been significantly smaller than those in the other three categories. The combined mean data from the 1987 nursery experiments when 25 different mother tree seedlots were used are shown in Table 2. Families 7 and 3 (c.f. Fig. 1) were two of the 25 families.

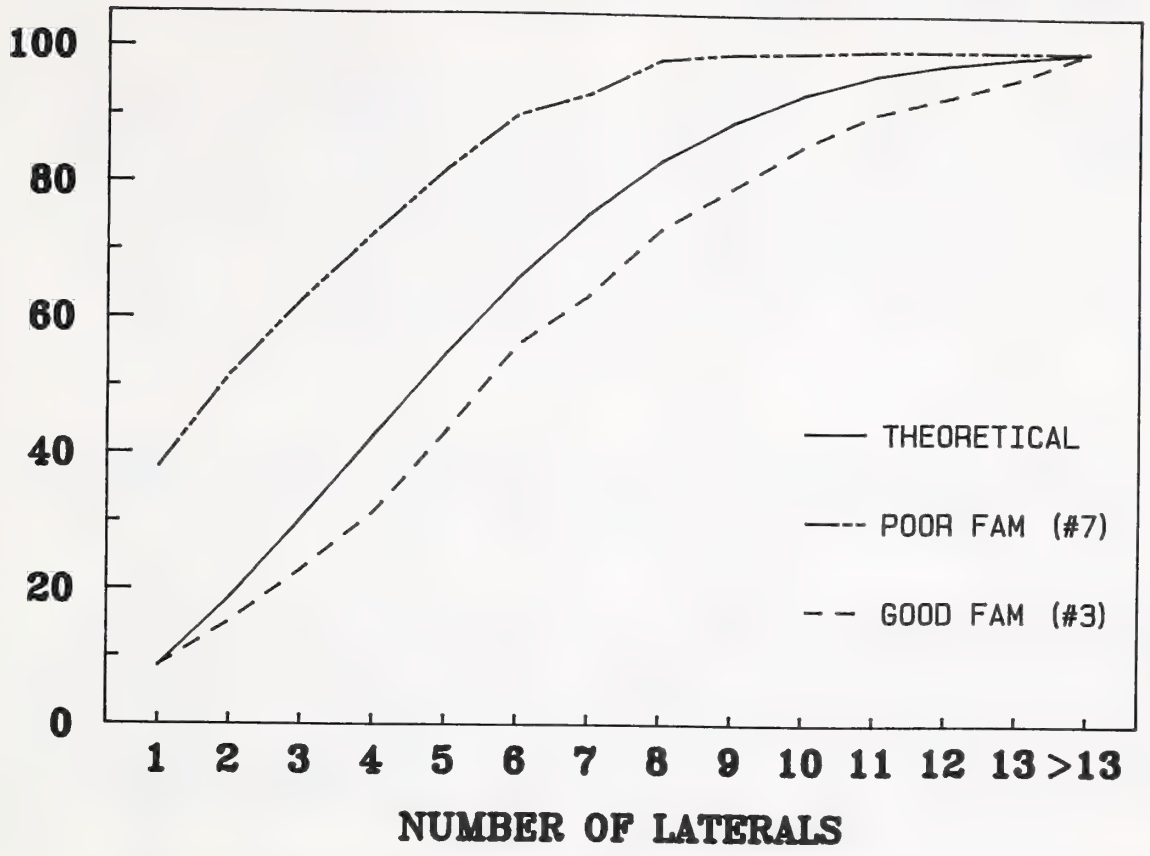

Figure 1. Theoretical frequency distribution of loblolly pine seedlings based on counts of first-order lateral roots compared with distributions developed from seedlots from a good (#7) and a poor (#3) mother tree.

Seedlings from the 1987 trial were quite large because they were not sheared in the nursery bed to conform to some predetermined optimum size. Even those in our 0-3 FOLR category would, on the average, qualify as Wakeley (1954) grade 2 seedlings. Grade 2 seedlings are generally considered to be among the best ones produced. Under our test conditions, seedlings with 0-3 FOLR have proven to be noncompetitive against their own siblings, even under ideal nursery growing conditions.

These differences in seedling quality evaluation based on different criteria illustrate the difficulty encountered in judging seedling quality based upon stem morphology. The obvious measures of "stem morphology" are height, which is easily obtained and maintained through fertilization and top shearing, and RCD. What of the other seedling morphological characteristics common to what Wakeley (1954) considered his "cull" or class 3

Table 1. Evaluation of loblolly pine seedlings from 25 families after lifting from the nursery beds.*

Number of lateral roots	Height (cm)	Root-collar diameter (mm)	D^2H (cm^3)	Average number of lateral roots	Percent of total seedlings
0-3	38.4b**	3.8d	6.25d	1.47d	39
4-5	46.5a	5.4c	14.06c	4.56c	20
6-7	49.1a	6.0b	18.64b	6.44b	17
$\geq$8	50.1a	7.1a	26.43a	9.96a	24

* Top weight, root weight, and total weight were determined by measurement of representative subsampled; each subsample contained 100 seedlings.
** Within columns, values followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

seedlings? How would the morphology of our large seedlings with few FOLR compare to Wakeley's cull individuals? Wakeley's cull seedlings were usually less than 12.7 cm tall; less than 0.32 cm in RCD; their stems were weak, often juicy and lacking mature bark. Primary single needles with few mature needles in fascicles and often having a bluish color; terminal buds were rarely present. These undesirable morphological characteristics, other than height and RCD, were common to a high percentage of seedlings in the 0-3 FOLR category in all tested families.

These various morphological traits, common to the least desirable individual seedlings of radically different sizes, appear to have biological implications worth considering in assessing other mother tree attributes. It appears that poor development of FOLR may just be a part of the genetic makeup of individual seedlings that is also expressed in the other questionable morphological attributes. Thus, a smaller percentage of progeny with few FOLR would be a valuable asset for mother trees to have when combined with other desirable characteristics.

If there is no biological implication to FOLR development, then our failure to identify quality seedlings before they have performed in the field will continue to be a problem and source of frustration. However, predictability of FOLR development coupled with the other undesirable traits, suggest that some standard nursery practice can be readily developed that could be useful for assessing selected mother trees before including them in seed orchards.

Operational Nursery Observations

The nursery seedlings used in this research were randomly selected from the nursery beds. The seedlings were grown under a variety of soil conditions, fertility regimes, and seedling bed densities. Some of the

nursery beds were not sheared but other beds were sheared either once or twice in late summer. Seedlings in some beds were root pruned; others were not.

Shearing has an impact on seedling stem morphology but may not significantly impact the FOLR frequency distribution. In general, the sheared seedlings' last growth increment lacked needles in fascicles and few had developed terminal buds when they were lifted. Since mowing was used to control height, seedlings with few FOLR were generally similar in height to those seedlings with many FOLR, but RCDs were somewhat smaller. In our experimental nursery, seedlings with few FOLR could generally be identified by a combination of seedling height and needle characteristics. This was not the case following shearing since many seedlings had both primary foliage and mature needles in fascicles.

Roots were undercut in different nurseries for different purposes. Sometimes roots were undercut to stress the seedlings, stopping height growth and inducing bud development that was delayed because of shearing. Other nurserymen sheared to improve root fibrosity. In either case, stressing of loblolly pine seedlings in the fall can severely impact carbohydrate translocation and metabolism in roots and thus impact diameter of FOLR. However, a single undercutting in early fall after terminal bud set does not appear to adversely affect FOLR development and significantly improves root fibrosity and feeder root development. We have not observed responses of siblings to undercutting since the few seedling lots examined were of mixed seedlot origin. However, if undercutting is used to stimulate terminal bud set on sheared seedlings, we might expect that FOLR frequency distribution of seedlings would be affected.

The only nursery growing condition in which the seedlings' observed FOLR frequency distribution consistently differed from the theoretical one was that related to high seedbed density. Seedlings grown at high bed densities had fewer FOLR and their roots were uniformly smaller. High density even appeared to impact the depth to which the taproot penetrated. Most nurseries did not have excessively high densities. The problem in evaluating the operational seedlings was the sporatic occurrence of excessive bed densities. High seedbed densisties also impacted stem morphology in a predictable pattern, making assessment of quality based on stem characteristics somewhat difficult. Seedbed densities in excess of ca $320/m^2$ generally resulted in a significant increase in the proportion of seedlings with 0-3 FOLR. We feel this reduction in FOLR size was a direct result of competition as few mature needle fascicles developed on the lower portion of the stem when stem density was too high.

CONCLUSIONS

Within sibling populations, seedlings with the fewest FOLR are the poorest competitors in the nursery. Our seedlings having few FOLR, although three times larger than Wakeley's (1954) grade 3 culls, share many undesirable morphologial traits: primary needles, succulent stems lacking

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mature bark, and absence of terminal buds. On the average, approximately 20 to 35% of the seedlings in most seedling populations share these undesirable traits, but some mother tree progeny have twice this number. Since seedling competitive position in the nursery is correlated with FOLR numbers some sibling groups can be more competitive than others. This propensity of sibling groups to have better FOLR development could be useful in obtaining a higher percentage of competitive seedlings in each nursery crop. Since FOLR development apears to be highly heritable, it may be useful in assessing selected mother trees for placement in seed orchards.

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Tony Squillace Award Winner
Best Paper

EARLY SELECTION OF LOBLOLLY PINE FAMILIES BASED ON SEEDLING SHOOT ELONGATION CHARACTERS

Bailian Li, S. E. McKeand, and H. L. Allen [1/]

Abstract.-- Seedlings of 23 open-pollinated loblolly pine (Pinus taeda L.) families and three checklots were grown in a greenhouse under two nitrogen (N) levels (5 and 50 ppm) for four months. Significant family differences were detected for the number of growth cycles, summer shoot elongation, number of stem units, and total seedling height. Positive correlations were found between family mean seedling height and 12-year height performance levels in the field, and correlations increased substantially after seedlings had set the first terminal bud. Summer shoot growth, cycle numbers, and number of stem units showed stronger correlations with field performance than seedling height. The correlations were consistently higher under the low N than under the high N condition. The height growth of 12-year loblolly pine families can be more accurately predicted by measuring summer shoot elongation of seedlings grown under the mimicry low N environment.

Keywords: Pinus taeda L., juvenile-mature correlation, summer shoot elongation.

INTRODUCTION

Tree breeders have recognized that tree improvement progress can be speeded up by early testing and selection if certain genetic relationships exist between juvenile and mature trees. The judgment is often made on growth rate alone without consideration of other nutritional and physiological characteristics. Research has confirmed large genetic variation in seedlings of tree species for physiological characteristics such as photosynthetic rate (Ledig and Perry 1967, Zelawski 1976, Ledig and Clark 1977), and uptake and use of nutrients (Jahromi et al. 1976, Johnson 1984, Kleinschmit 1982). Some success has been made to relate the morphological and physiological characteristics (Cannell et al. 1978, Waxler and van Buijtenen 1981) and nutritional traits (Cotterill and Nambiar 1981) with genetic potential of field growth. Family seedling shoot elongation patterns were reported to be strongly associated with 8-year height performance in the field (Bridgwater et al. 1985, Williams 1987). The height after free growth of seedlings was reported to have strong genetic association with height growth in the field (Williams 1987). A better understanding of shoot morphology in controlled environmental conditions which mimic major environmental variables in the field may be important for successful early selection (Cannell et al. 1978, Bridgwater et al. 1985).

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N deficiency is usually a limiting factor for tree growth in most forest stands, so successful juvenile selection may depend on a better understanding of various physiological processes involved with the growth under the mimicry low N conditions. Seedling growth under the mimicry low N condition may be better correlated with field performance. This study was designed to examine the genetic variation in seedling shoot growth under two contrasting N environments (N-stressed and high N), and to determine the relationship between seedling growth and 12-year height performance in the field. It was part of a larger study designed to evaluate genetic variation in N use efficiency, carbon allocation, root characters, and shoot growth patterns under low and high N, and to use these traits for early selection.

MATERIALS AND METHODS

Twenty-three open-pollinated loblolly pine families from the northern coastal plain of North Carolina and southern coastal plain of Virginia were used in the study. All families have been extensively evaluated in long-term genetic tests of the N. C. State University Tree Improvement Cooperative. In addition, open-pollinated seeds of 7-56, a consistently good family from the coastal plain of South Carolina, a bulked seed lot from unimproved stands (commercial check), and a bulked seed lot from coastal seed orchards of North Carolina were also included in the study as check lots.

A split-plot design with N treatments as whole plots and loblolly pine families as sub-plots was replicated in four blocks. Three seedlings per families were randomly distributed within each treatment-block combination. Seeds were weighed, stratified, and sown in flats, then seedlings were transplanted to 6x6x16" square pots filled with a sterilized sand medium. N treatments were delayed until the seedlings were four months old in order to avoid possible seed size or other maternal effects on early growth (Cannell et al. 1978). The two N levels, 5 and 50 ppm (equal proportion of NH_4 and NO_3), were applied about three times per week depending on leaf water potential measurements (Li 1989). Predawn leaf water potential was monitored with a pressure chamber and nutrient solutions were supplied when the leaf water potential of -0.5 MPa was reached.

Seedlings were harvested after four months of N treatments. The height, cycle number, and length of cycles were measured. Summer shoot elongation was determined by subtracting first cycle length from the total seedling height. A mixed linear model with N treatment effect fixed and family effect random was used for evaluation of experimental data (Li 1989). The General Linear Model (GLM) procedure in Statistical Analysis System (SAS) was utilized to analyze the data (SAS Institute 1982). Genetic variance components were estimated for half-sib families to obtain heritability estimates. Narrow-sense heritabilities were estimated for those shoot characters which showed significant family variation (Li 1989). Family mean seedling shoot characteristics were correlated with the height performance level (PL) values in the genetic tests. Performance level values were standardized values averaged over fifteen 12-year-old genetic tests of the North Carolina State University Tree Improvement Cooperative (Hatcher et. al. 1981). These tests were not balanced in terms of representing every full-sib family in the same test, but each of the 23 half-sib families was represented by an average of 17 full-sib crosses, and each family was tested in at least five tests in different environments.

RESULTS AND DISCUSSION

Family variation

There were significant family differences for all traits except the mean stem unit length at low N (Table 1). The number of stem units showed more variation than the mean stem unit length, which agrees with other studies that showed that the number of stem units accounts for most of the differences in shoot elongation for different conifer species (Bridgwater et al. 1985, Cannell et al. 1976, Kremer and Larson 1983, Kremer 1986). Heritability estimates for cycle numbers, summer shoot length, and the number of stem units were higher than those for seedling total height in both N conditions (Table 1). The number of growth cycles showed the strongest genetic control, while the mean stem unit length was under relatively weak genetic control. Heritability estimates from low N were higher than their corresponding estimates in high N. Standard errors for heritability estimates were usually less than half of the magnitude of heritability estimates, indicating that these estimates were reliable.

Table 1. Heritability estimates[1/] and their standard errors (in parentheses) for some shoot characteristics under low and high N conditions, and combined over the two N levels.

	Heritability (h^2)		
Traits	Low N	High N	Combined
Cycle Number	0.94 (0.02)	0.85 (0.33)	0.94 (0.05)
Total Height	0.53 (0.25)	0.37 (0.22)	0.21 (0.11)
Summer Growth	0.73 (0.30)	0.48 (0.27)	0.56 (0.22)
# Stem Units	0.74 (0.30)	0.44 (0.23)	0.58 (0.22)
Mean Stem Unit Length	----	0.20 (0.14)	0.15 (0.09)

[1/] Heritabilities were calculated only for traits showing significant family differences.

Correlations with 12-year Height

Positive correlations were found between family mean seedling height and 12-year height PL in the field (Figure 1a), and the correlations increased substantially after seedlings had set the first terminal bud at about 4-months from sowing. Summer growth had a higher positive correlation with 12-year height than total height (Figure 1b). Williams (1987) also reported that loblolly pine seedling height at the first bud was negatively correlated with 8-year height, but the correlation increased as seedlings grew in height.

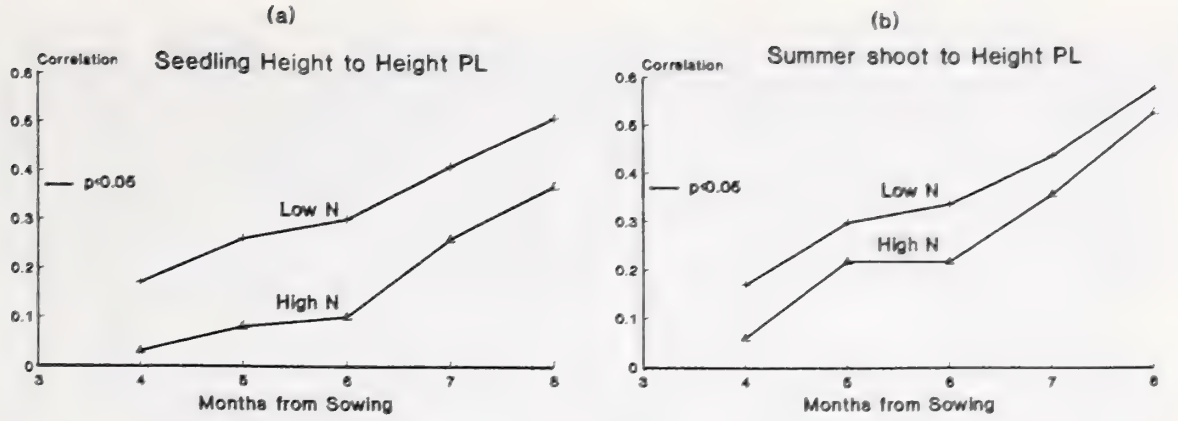

Figure 1. Family mean correlations of 12-year height PL with seedling height (a) and summer growth (b).

The low correlations in the early growing season may be influenced by seed weights. This was supported in part by a decrease in the correlation between the family mean seed weights and family mean seedling height as seedlings grew (Figure 2). Family mean seed weights were strongly correlated with seedling height at the end of free growth, but the correlations decreased after seedlings started to produce summer growth. The correlations between shoot growth traits and 12-year height PL increased as the proportion of summer growth increased over the experimental period (Figure 1). Cannell et al. (1978) also reported that positive correlations between seed weights and seedling height in loblolly pine seedlings disappeared once the seedlings exceeded about 140 mm in height. The correlation of seedling height with 8-year height increased after seed weight effects disappeared.

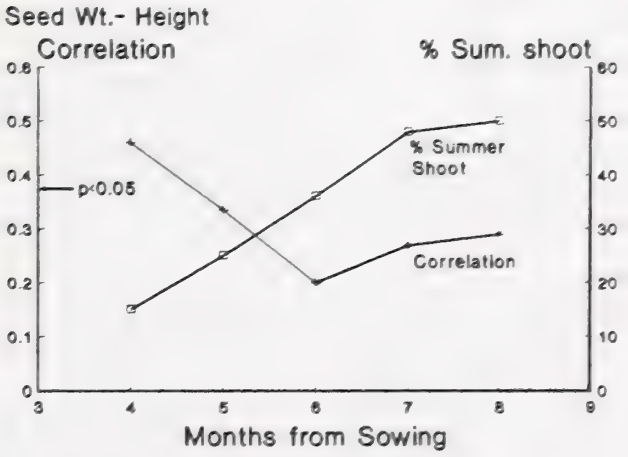

Figure 2. Family mean correlations between seed weight and seedling height over time. Also shown is the percent of total height which summer shoot growth contributes.

Mean summer shoot length, average number of growth cycles, and the mean number of stem units of 23 families showed stronger correlations with 12-year height PL in the field than seedling total height (Table 2). Families which produced more growth cycles and initiated more stem units per cycle had greater summer growth as seedlings (Li 1989), and also grew better in the field. The number of stem units had higher correlations with 12-year height than the mean stem unit lengths. The summer growth was the best predictor of 12-year height in the field. The precision of prediction could be further improved by measuring seedlings under the low N condition because the juvenile-mature correlations were consistently higher in low N than those in high N (Table 2). This supported the hypothesis that seedling families grown under low N are better correlated with field performance levels. Performance of known check lots (commercial check and 7-56) among families also supported this result. The commercial check (CC) and 7-56 had essentially the same height in the high N condition, but 7-56 produced 28% more height growth in the low N condition. Family 7-56 produced 39% more summer growth than CC at low N but only about 8% at the high N condition.

Table 2. Family mean correlations of seedling shoot characters with 12-year height performance levels for two N treatments.

Traits	Low N	High N	Combined
Total Height	0.47*	0.21	0.43*
Cycle Numbers	0.51*	0.43*	0.44*
Summer Growth	0.58**	0.48*	0.52*
# Stem Units	0.54**	0.35	0.47*
Mean Stem Unit Length	0.02	0.18	0.13

*, ** : Significantly different at $p<0.05$ and $p<0.01$, respectively.

The stronger correlation at low N indicated that 12-year height of loblolly pine families could be more accurately predicted from the seedlings grown under the low N condition than under the high N condition. The improvement in correlations by measuring seedlings under low N could be due to the influence of low N availability on family differences in 12-year height in the field conditions. In fact, the low N level in this experiment was similar to that in the field conditions in terms of N concentration in the tissues. N concentration of the seedling needles on the average was 0.9% in low N and 1.8% for high N. This was close to the average foliar N concentration (0.8 - 1.2%) reported for 2-year-old loblolly pine seedlings of non-fertilized plots across a range of sites (NCSFFC 1984). Thus, the low N (5 ppm) was considered as a mimicry N-stressed environment in the field, while the high N (50 ppm) was a N luxury environment which was not a common field condition for loblolly pine genetic tests. The efficiency of early selection is expected to increase under this mimicry low N condition because of higher heritabilities and juvenile-mature correlations.

Other studies also showed the possibilities for early selection under the mimicry environmental conditions. The growth rate of loblolly pine seedlings was shown to be significantly correlated to field stem volume of the same families at age 8 when seedlings were grown under similar moisture conditions as in the field (Cannell et al. 1978). Seedling height grown under growth-accelerating environments (high fertility and supplemental lighting) had no correlation ($r = 0.13$) with 8-year height, but seedlings grown under more natural day length environments showed strong correlations ($r = 0.59$) with 8-year height in the field (Williams 1988).

Based on these results and others (Bridgwater et al. 1985, Li 1989, Williams 1987), it seems that operational tree improvement programs can utilize these measures of seedling shoot elongation for early selection. First, this can be used to screen parents in the breeding population before crossing, or to eliminate the poorest crosses before field progeny testing. The cost of breeding and field progeny tests can be reduced substantially or more individuals of each cross can be tested in the field. Secondly, this procedure can be used to increase the precision of selections. The seedlings of the same families which have been tested in the field can be grown in the greenhouse for evaluation. A selection index could be used to combine field information and greenhouse evaluation for selection. Furthermore, the combination of the shoot elongation traits with other seedling characters (root morphology and N use efficiency) showed promise to further increase the precision of predication (Li 1989).

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VARIATION OF SLASH PINE WOOD SPECIFIC GRAVITY IN DIFFERENT PROGENY TEST ENVIRONMENTS

G.A. Lowerts and M.H. Zoerb, Jr.[1/]

Abstract.--In 1973, 28 open-pollinated first generation seed orchard slash pine families originating from Florida and Georgia wild stand selections were planted in progeny tests at two locations; Effingham County, Georgia and Putnam County, Florida. Eight families were selected for wood specific gravity analysis in 1987 (age 15). All families displayed significantly higher specific gravity at the Florida location than at Georgia location. The average specific gravity of families originating from Florida selections was significantly greater than that of families originating from Georgia selections at both locations. Family specific gravity appeared to be stable across locations.

Keywords: Pinus elliottii, wood specific gravity, geographic variation.

INTRODUCTION

Open- (o.p.) and control-pollinated slash pine (Pinus elliottii var elliottii Engelm.) progeny tests have been established by many forest products companies. The inheritance of slash pine wood specific gravity has been shown to be under strong genetic control with narrow sense heritabilities (h^2) ranging from 0.43 to 0.68 (Squillace, et al. 1962; Goddard and Cole 1966; Sohn and Goddard 1975). Although several studies have estimated the heritability of specific gravity, the trends in specific gravity among geographic sources and the stability of family mean specific gravity at different progeny test locations has not been explored to any great extent. In natural stands, slash pine specific gravity increases from north to south (Zobel and Talbert 1984; Megraw 1985); however, Goddard and Cole (1966) determined that no trend in specific gravity variation was apparent among geographic sources in one six-year-old progeny test.

The objective of this study was to determine the variation and stability of specific gravity in open-pollinated progeny of high and low gravity first generation seed orchard ortets at two different locations within the species range.

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METHODS

In 1973, 28 open-pollinated families, from a first generation seed orchard, and two unimproved bulk seedlots were planted in four replications of ten tree row plots at two locations. Location one (poorly drained Olustee soil type) is in Effingham County, Georgia and location two (poorly drained Leon soil type) is approximately 150 miles south in Putnam County, Florida. Site preparation of the Georgia and Florida locations consisted of disk and bed and chop and bed, respectively, in 1972. No other cultural treatment other than mowing prior to measurement has been applied to either site.

Ideally, 20-30 individuals per family should be used for specific gravity analysis (Jett, 1989). Due to the poor survival (<55%) and high incidence of stem fusiform rust (Cronartium quercuum Miyabe ex Shirai f. sp. fusiforme (Cumm.) Burds et Snow) (>80%) infection at the Georgia test location (age 12 data), only eight individuals with rust free stems per family were available for specific gravity analysis. Based upon geographic source and the constrictions of survival and stem rust incidence, four families from Florida selections with relatively high specific gravity (mean 0.64) and four families from Georgia selections with lower specific gravity (mean 0.53) were available for analysis at both locations. Specific gravity of the original select tree was previously determined at the time of selection for seed orchard use by various agencies using the water displacement method (Smith 1954).

Regardless of the replication, the best (i.e. dominant or codominant) eight trees per family (n=8) were felled at each location (64 trees/location). Total height and breast height diameter were measured on each tree prior to felling. A wood disk, approximately 1" thick, was cut at breast height from each tree. Two wedges (usually 180^{o} apart) of clear wood were cut from the pith to the bark. The unextracted specific gravity of the whole wedge was determined using the water displacement method. The specific gravity value at breast height for each tree was determined by mean of the two wedges.

Statistical Analysis

Family mean, and standard deviation of specific gravity, height, and diameter were calculated using individual tree data. Location, geographic source, location X source, and family within source are the only sources of variation present in this study. Since a true replication effect was not available, individual trees cannot be used as a source of variation.

Analysis of variance procedures using family mean values were performed on a mixed model with the following main effects: location, source, location X source, and family within source. Location and source were considered to be fixed effects while family was considered to be a random effect.

RESULTS

At age fifteen (1987), total tree height was significantly ($p<0.01$) different between locations (Table 1). The mean height of the eight families at the Florida location (48.4 ft) was greater than the Georgia location (43.9 ft). Diameter (DBH) was not significantly different between locations (Table 1). There was no significant difference between sources, and among families within source for height and diameter (Table 1). Also, the location X source interaction was not significant (Table 1). In comparison with the Florida location, the poor growth and high incidence of rust infection (80% at age 12) of the slash pine families at the Georgia location strongly suggests that slash pine is poorly suited for operational plantations near the northern limit of the species range.

Mean specific gravity of all families was significantly greater ($p< 0.01$) at the Florida location (0.552) than at the Georgia location, (0.510). At both locations, the families from the Florida source possessed a significantly ($p<0.05$) greater mean specific gravity than the families from the Georgia source (Table 1). The mean specific gravity of Florida source families exceeded that of Georgia source families by 4% at the Georgia and by 3% at the Florida locations (Table 2). Without exception, individual family mean specific gravity was greater at the Florida location than at the Georgia location (Table 2). Small rank order changes were evident between locations. For example, family 10-287 (Florida source) displayed the highest mean specific gravity (0.539) at the Georgia location but only the fourth largest mean value (0.555) at the Florida location (Table 2). However, extreme rank order change, such as moving from first to last place, was not evident. The location X source interaction was not significant, and there was no significant difference among families within source (Table 2). The results of this study were strongly influenced by the small test size.

DISCUSSION

Over all families, total tree height, diameter, and specific gravity were greater at the Florida than at the Georgia location. Since specific gravity is influenced by various hormones produced by the crown, most environmental factors that impinge on crown vigor will affect specific gravity (Megraw 1985). Favorable environmental conditions such as increased summer rainfall, a longer growing season, etc., have been suggested as possible reasons for natural stands of Pinus spp. to display increasing specific gravity from north to south and west to east (Megraw 1985; Zobel and Talbert 1984). Environmental conditions apparently favor

Table 1. Age fifteen combined location analysis of variance for specific gravity (S.G.), total tree height, and diameter (DBH).

Source	DF	S.G.	Height	DBH
		----------Prob. F----------		
Location	1	0.0004**	0.0009**	0.6251
Source	1	0.0331*	0.2035	0.4145
L X S	1	0.8333	0.8367	0.5895
Family(S)	6	0.9364	0.5937	0.4607
Error	6			

Where **=significant at the 0.01 level of probability
*=significant at the 0.05 level of probability

Table 2. Age fifteen (1987) mean specific gravity, total tree height, and diameter (DBH) by location, family, and source within location.

Family	Source	Specific Gravity	DBH (in.)	Ht (ft.)
Effingham County, Georgia				
10-279	Florida	0.504	5.5	41.4
10-286	Florida	0.521	6.9	45.9
10-287	Florida	0.539	6.7	43.8
10-373	Florida	0.512	7.0	46.3
10-223	Georgia	0.504	6.5	42.9
10-227	Georgia	0.494	6.4	43.9
10-264	Georgia	0.510	6.0	43.3
18-29	Georgia	0.498	6.2	43.8
	Location Mean	0.510	6.4	43.9
Florida	Source Mean	0.519	6.5	44.3
Georgia	Source Mean	0.501	6.3	43.4
Putnam County, Florida				
10-279	Florida	0.562	6.5	49.3
10-286	Florida	0.559	6.5	47.9
10-287	Florida	0.555	6.4	49.3
10-373	Florida	0.563	6.7	49.3
10-223	Georgia	0.538	6.3	46.4
10-227	Georgia	0.550	6.5	49.1
10-264	Georgia	0.537	6.6	48.6
18-29	Georgia	0.554	6.5	47.0
	Location Mean	0.552	6.5	48.4
Florida	Source Mean	0.560	6.5	48.9
Georgia	Source Mean	0.544	6.5	47.8

1/ Family Means N = 8
Source Means N = 4
Location Means N = 8

the development of higher specific gravity slash pine at the Florida location. The greater specific gravity of all families at the Florida location agrees with the observed trend in natural stands.

The families originating from the Florida source displayed a greater mean specific gravity at both locations. Source effects were consistent between locations and followed the specific gravity trend observed in natural stands. In contrast, Goddard and Cole (1966) did not observe a trend among geographic sources of slash pine in a six-year-old progeny test. However, their study was conducted at one location using young trees. Perhaps, the presence of mature wood in this study allowed for the expression of the geographic trend in specific gravity. Several studies in loblolly pine have shown natural stand geographic trends are not always repeated in provenance tests at various locations (Byram and Lowe 1988; Saucier and Taras 1967), but other studies (Jackson and Strickland 1962) did observe increased specific gravity from southern loblolly sources in a provenance test of several sources in Georgia. Further examination of specific gravity trends among geographic sources of slash and loblolly pine at multiple locations is needed.

Individual family specific gravity of both sources was consistently greater at the Florida location and appeared to relatively stable with minor rank order changes. The stable performance of progeny from high specific gravity parents across many locations has also been reported in loblolly pine (Anonymous 1989; Byram and Lowe 1988). Of all the characteristics studied in Pinus spp., specific gravity is under the strongest degree of genetic control and offers the greatest opportunity for manipulation (Zobel and Talbert 1984). The stability of progeny from high specific gravity sources at different locations is a reflection of strong genetic control. Since environmental factors were not examined and because of the small sample size in this study, additional testing with an increased number of locations, families, and individuals within family will be necessary to confirm the consistency of source effects and the stability of families across locations.

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CONCURRENT SESSION: 1B

COMPARISON OF PLOT DESIGNS FOR SHORT-TERM GENETIC TRIALS

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Abstract.--Consistant reports from retrospective early genetic trials suggest height or height increment measured in closely-spaced trials can be used to screen families by age 3-years. Closely-spaced trials come into intense tree-to-tree competition and competitive stress may bias selection and parameter estimates. In our closely-spaced plot design study, bias from inter-family competition was not apparent, even when families from different seed sources were interplanted. Block plots were found to be unnecessary, row and multiple single-tree plots provided similar mean family rankings and multiple single-tree plots appeared slightly more efficient in short-term genetic trials.

Keywords: Inter-family competition, genetic parameter estimates, family by plot design interactions, *Pinus taeda* L.

INTRODUCTION

There are increasing reports of high positive correlations between seedling height and mature-tree height (Franklin 1983, Campbell *et al.* 1985, Magnussen and Yeatman 1986, Williams 1987, Stonecypher pers. comm.). The trend among some species, including loblolly pine, is for juvenile-mature correlations to become increasingly positive as seedling height increases (e.g. Fig. 1). This trend from negative to positive correlations has been attributed to substantial changes in seedling ontogeny (Williams 1987).

Most reports come from studies planted on agricultural sites where intensive site preparation has improved progeny testing resolution. The best results have been achieved with a minimum square spacing of 33 to 50 cm; a survey of North Carolina loblolly families indicates age-age correlations are negligible at spacings lower than 10 cm (Wearstler 1979, Fig. 1). Close spacing reduces area required for testing and site heterogeneity (Rehfeldt 1983); it may also aid trait expression in other ways (Franklin 1983).

These closely-spaced tests experience intense tree-to-tree competition early and there is a genetic component to competition which may bias genetic parameter estimates and selection (Adams 1980, Shimizu 1985, Tuskan and van Buijtenen 1986). Competition among genetic entries is likely to increase the risk of making incorrect selections in closely-spaced short-term trials. Gain estimation may also be biased by intense inter-family, inter-genotypic competition.

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Planting families in blocks reduces bias from inter-family competition but at a cost. Block plots require more replications to achieve the same degree of resolution as row or single-tree plots. Increasing the number of replications within a test substantially increases testing costs and may increase site heterogeneity. Can smaller plot designs in closely-spaced short-term genetic trials be used without biasing selections? Our objective is to compare plot designs from a closely-spaced study for similarities in mean family ranks and genetic parameter estimates.

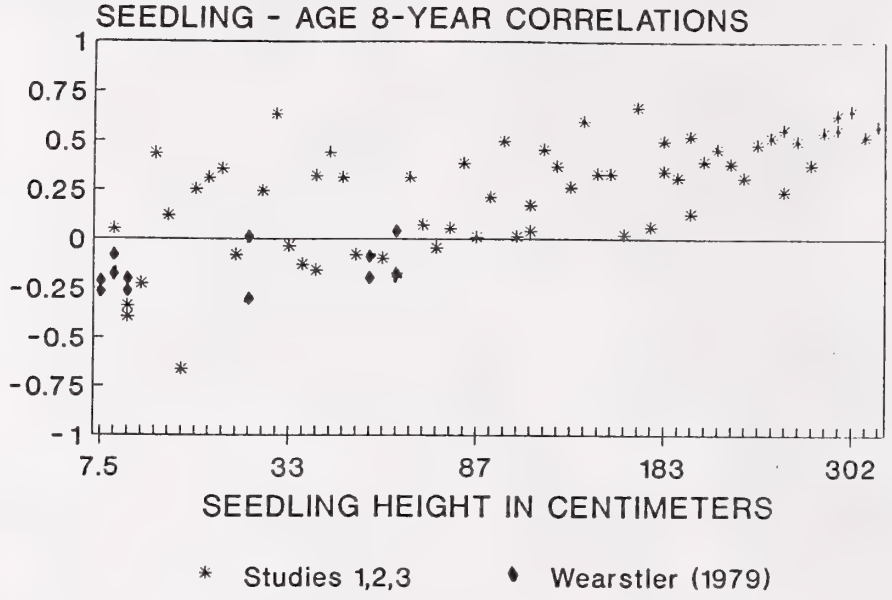

Figure 1. Retrospective early testing studies using Weyerhaeuser's first-generation open-pollinated families. All tests were planted on agricultural sites at close spacings. Data were used from Wearstler (1979), Franklin (1983), Williams (1987) and an unpublished Weyerhaeuser study. Eighth-year field test data are described in Lambeth and Duke (1981).

MATERIALS AND METHODS

In March 1980 a study was installed in five replications of four plot designs: 60 x 60 cm row, 60 x 60 cm multiple single-tree (a.k.a. non-contiguous), 60 x 60 cm block and 120 x 120 cm row plots; each containing nine measurement trees on an agricultural site located at Magnolia, Arkansas. The nine measurement trees in the block plots were surrounded by two buffer rows which were not measured. The terminal trees at the end of each row plot were not used in the analysis. To obtain equal sample size two trees were randomly discarded from each multiple single-tree and block plot.

Height was measured in December 1981, November 1982 and December 1983. Height was used to detect the onset of competition by comparing growth in the 120 x 120 cm and 60 x 60 cm row plots. The 120 x 120 cm plots were not used in any other analysis. Plot means from the 60 x 60 cm plots were used 1) to estimate Pearson's product-moment correlation coefficients and 2) in family by plot design factorial analysis of variance based on the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_k + \alpha\gamma_{ik} + \beta\gamma_{kj} + \alpha\beta\gamma_{ijk} \quad (1)$$

where, μ = overall mean,
α_i = replication effects, i = 1,2,....r,
β_j = family effects, j = 1,2,.....f, and
γ_k = plot design effects, k = 1,2,.....p.

Individual tree values were used to estimate variance-components and intra-class correlations for each plot design based on the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{k(ij)} \quad (2)$$

where, μ = overall mean,
α_i = replication effects, i = 1,2,.....r,
β_j = family effects, j = 1,2,.....f, and estimates 1/4 additive genetic variance, and
$\varepsilon_{k(ij)}$ = within plot error, k = 1,2,.....s for each ij, and estimates environmental variance, 3/4 additive genetic variance and dominance genetic variance.

Phenotypic variance was estimated as family variance + within-plot variance + family by replication variance.

Families represented half-sib, open-pollinated female families from five separate seed sources. There were seven families from coastal North Carolina, three from Piedmont, North Carolina, three from north Louisiana/south Arkansas, one from north Mississippi and one from east Texas. These seed sources were intentionally selected to exaggerate differences in growth and inter-family competition.

RESULTS

Inter-family competition began influencing seedling growth between the second and third measurement dates based on height growth of the 120 x 120 cm versus 60 x 60 cm row plots (Table 1). Harms and Langdon (1976) likewise reported height reductions across similar spacings.

Differences among families and among 60 cm square plot designs were significant at each measurement date (Table 2). Thus, differences among families were detectable in all plots and plot design influenced the absolute expression of mean family height. Row plots produced the tallest trees, followed by multiple single-tree and then block plots (Table 1). In contrast, Hart (1986) found no difference in height among various plot designs at conventional spacings. The differential performance of the plot designs tested in the close-spaced study merits further study, yet in reference to early genetic trials, plot design did not affect mean family rank. There were no significant family by plot design interactions for total height or third-year incremental height at the 60 x 60 cm spacings (Table 2).

The absence of family rank changes across plot designs was confirmed by Pearson's product-moment correlation coefficients. Mean family heights were

positively correlated among all plot designs at each measurement date (Table 3). In general, the correlation coefficients increased between the first and second measurements, then decreased slightly at the third measurement. The decrease in the correlation coefficients at the third measurement date corresponds to the time when competitional effects were detected for height.

Table 1. Height over time by plot design for multiple single-tree, row and block plots containing 15 open-pollinated families representing 5 seed sources of loblolly pine grown at close spacings on an agricultural site.

Plot Design	Planting Height	1st-Year Height	2nd-Year Height	3rd-Year Height
	meters			
120 x 120 cm Row	0.18 a[1/]	0.46 a	1.76 a	3.16 a
60 x 60 cm Row	0.17 a x[2/]	0.46 a x y	1.76 a x	3.04 b x
60 x 60 cm Block	0.17 x	0.45 y	1.70 y	2.96 y
60 x 60 cm Multiple Single-tree	0.17 x	0.47 x	1.73 x y	2.99 x y

[1/] Means within columns for 120 x 120 cm and 60 x 60 cm row plots connected by a similar letter are not significantly different based on a t-test at alpha $\leq$ 0.05.

[2/] Means within columns for 60 x 60 cm plots connected by a similar letter are not significantly different based on Waller-Duncan's test at alpha $\leq$ 0.05.

Table 2. Partial R^2 from family by plot design factorial analysis of variance for plot mean height over time.

Source of Variation	df	1st-Year Height	2nd-Year Height	3rd-Year Height	3rd-Year Increment
Replication (R)	4	.171	.155	.213	.291
Plot Design (P)	2	.025*	.018*	.016*	.007
R x P	8	.103	.081	.098	.110
Female (F)	14	.171**	.221**	.229**	.132**
R x F	56	.135	.154	.115	.097
P x F	28	.075	.054	.069	.073
Error	112	.318	.318	.260	.290

* and ** indicate significance based on F-test results at alpha $\leq$ 0.05 and 0.01, respectively.

Table 3. Pearson's correlation coefficient (r) among plot designs for family mean height over time.

	1st-Year Height	2nd-Year Height	3rd-Year Height	3rd-Year Increment
Multiple Single-tree vs. Row	.62[1/]	.82	.71	.73
Multiple Single-tree vs. Block	.66	.68	.54	.53
Row vs. Block	.56	.69	.69	.70

[1/] r-values $\geq$ .51 and .63 are significantly greater than zero at alpha $\leq$ 0.05 and 0.01, respectively.

The magnitude of the variance estimates for additive genetic, error (within-plot) and total phenotypic variances varied among plot designs. At the first measurement date, additive variance estimates were largest for the multiple single-tree plots, and at the third measurement date additive variance estimates were largest for the block plots. The multiple single-tree plot had the largest error estimates of the three plot designs at each measurement date. Estimates of error variances for the row and block plots were similar. The difference in the size of the estimates of error variances between multiple single-tree and row or block plots were less at close-spacings than that reported by Loo-Dinkins and Tauer (1987) at conventional spacings.

Changes over time in parameter estimates were generally similar among the three plot designs (Table 4). For example, estimates of phenotypic variances increased over time in all plot designs. These results differ from Franklin (1979) in that additive variance estimates did not decrease after competition began affecting growth.

As a consequence of plot differences in variance estimates, intraclass correlation estimates also varied. Intraclass correlation estimates were initially highest for the multiple single-tree plot, yet at the third measurement date intraclass correlation estimates were similar among plot designs (Table 4).

The changes in means and variances suggested the error variances may not be homogeneous among plot designs, and that a logarithmic transformation may be necessary. The partitioning of inter-family competition variance among the sources of variation also varied among plot designs (McCutchan pers. comm.), and further supported the hypothesis that error variances may not be homogeneous. Using Bartlett's test, the probability of heterogeneous variances was greater than .05 but less than .10; thus, transformations were not necessary.

Table 4. Estimates of genetic parameters for height over time derived from three plot designs at close spacing on an agricultural site.

Plot Design	1st-Year Height	2nd-Year Height	3rd-Year Height
Multiple Single-tree			
Error Variance	.014	.088	.133
Additive Variance	.006	.023	.060
Phenotypic Variance	.018	.117	.201
Intraclass Correlation	.083	.049	.075
Row			
Error Variance	.012	.072	.123
Additive Variance	.002	.018	.048
Phenotypic Variance	.014	.087	.156
Intraclass Correlation	.036	.052	.077
Block			
Error Variance	.012	.077	.126
Additive Variance	.001	.030	.062
Phenotypic Variance	.061	.107	.174
Intraclass Correlation	.004	.070	.089

DISCUSSION

Varying degrees of inter-family competition across plot designs had no effect on mean family performance. Results are limited to three years of data from a closely-spaced trial, and consequently the prolonged, cumulative effects of inter-family competition were absent. Differential height measurements between 120 cm and 60 cm plots were detected only in third-year height.

Plot design had no effect on the family mean ranking. Morrow (1983) likewise found no significant seed source by plot design interactions for row and block plots at conventional spacings. Conversely, Hart (1986) found mean family ranks were not correlated between block and row plots or block and multiple single-tree plots, but were correlated between row and multiple single-tree plots. There were minor changes in family ranks among plot designs that did not influence the significance of the correlations in our study. These changes in family rank may be due to measurement precision, measurement scale or an artificial designation of a culling level.

Loo-Dinkins and Tauer (1987) reported higher statistical efficiencies with multiple single-tree plots compared to row or block plots, even on uniform sites. Lambeth *et al.* (1983) concluded fewer replications are required for multiple single-tree plots because of these higher efficiencies. The statistical efficiency gained from multiple single-tree plots in long-term genetic trials is partially negated by planting early genetic trials on agricultural sites at close spacing for a short period of time. Site uniformity, close spacing and short duration studies diminish

some of the advantages of multiple single-tree plots. Therefore, the difference in the number of replications required for similar efficiencies between multiple single-tree and row or block plots is less in short-term genetic trials than in tests planted at conventional spacings.

In conclusion, competition adversely affected growth, yet there were no appreciable changes in family ranks across plot designs at 60 cm square spacings. Selection based on third-year height or third-year incremental height will not be biased by inter-family competition. Block plots appear unnecessary in closely-spaced, short-term trials and should be superceded by row or multiple single-tree plots.

ACKNOWLEDGMENTS

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EARLY TESTING PROCEDURES APPLIED TO TREE IMPROVEMENT PROGRAMS

W. J. Lowe and J. P. van Buijtenen[1/]

Abstract--Step-wise testing can be used to incorporate early testing procedures into operational tree improvement programs. By rejecting poor performing families after each test, field testing efficiency can be improved. Traits suitable for early testing procedures can be 1) important juvenile traits, 2) mature traits that can be evaluated at juvenile age and 3) a juvenile trait that is correlated with an important mature trait. Complementary mating schemes and positive assortative mating can be incorporated to increase genetic gains. The use of early testing procedures in the Western Gulf Forest Tree Improvement Program's slash pine, loblolly pine and longleaf pine improvement programs is discussed.

Keywords: Step-wise testing, complementary mating, assortative mating, Pinus taeda L., Pinus elliottii Engelm., Pinus palustris Mill.

INTRODUCTION

Tree improvement programs typically have long breeding cycles. The time delay between grafting and flower induction and the time required for progeny testing are the major factors causing the length of the breeding cycle. Research regarding fertilization, drought stress and the use of plant hormones have identified techniques that can be used to reduce the flower induction phase. Lambeth et al. (1983) showed that estimates of rotation age performance for loblolly (Pinus taeda L.) can be obtained at earlier ages than one-half of the projected rotation. Most of the selection work in southern pines is currently done between the ages of five and 10 years.

Selections have been made in the nursery (Zobel et al. 1957, La Farge 1975), and on the basis of greenhouse or growth chamber studies (Cannell et al. 1978, Waxler 1980, Miller 1982 and Davison 1984) in efforts to reduce the selection age. Field performance has not been

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consistently predicted on the basis of these studies.

When early testing procedures are developed, techniques are needed to incorporate them into operational tree improvement programs. A pilot-scale, early-testing study with loblolly pine was described by van Buijtenen et al. (1986). They used a step-wise screening process to evaluate a number of different traits in the laboratory. Lowe and van Buijtenen (1989) describe the procedures incorporated by the Western Gulf Forest Tree Improvement Program (WGFTIP) to utilize early testing techniques for growth in the loblolly pine breeding program.

This paper discusses the opportunities for early testing procedures and the techniques to incorporate these procedures into operational tree improvement programs, using the procedures adapted by WGFTIP as an example.

EARLY TESTING

Early-testing procedures could be used on three major types of traits as follows: 1) a juvenile trait that is important - an example of this is first year survival; 2) a mature trait that can be evaluated at a juvenile age - resistance to fusiform rust is an example of this type of trait and 3) a juvenile trait that is correlated with a desirable mature trait - seed or seedling characteristics that correlate with volume production in the field typify this group of traits.

Early testing procedures can result in two types of errors (Figure 1). A family with good field performance could be rejected on the basis of early test data (type A error). When a family with good early test performance fails to perform well in later field tests, the second type of error occurs (type B error). If the early testing procedure is intended to select a few outstanding families or reject a few poor families, these types of errors assume different levels of importance.

In an effort to reduce the breeding cycle, most early testing studies have concentrated upon selecting a few outstanding families. In this approach, both types of errors are important because families with poor field performance can be selected and families with good field performance can be rejected. Only the type A errors (rejection of families with a good field performance) are important if the objective of the early testing procedure is to reject a few poor families. The families with poor field performance (type B errors) will be identified in later field tests.

To incorporate early testing procedures into operational tree improvement programs, the concepts of step-wise testing and complementary breeding and testing schemes are very useful. Step-wise testing involves subjecting the seedlings to a series of tests. After each test

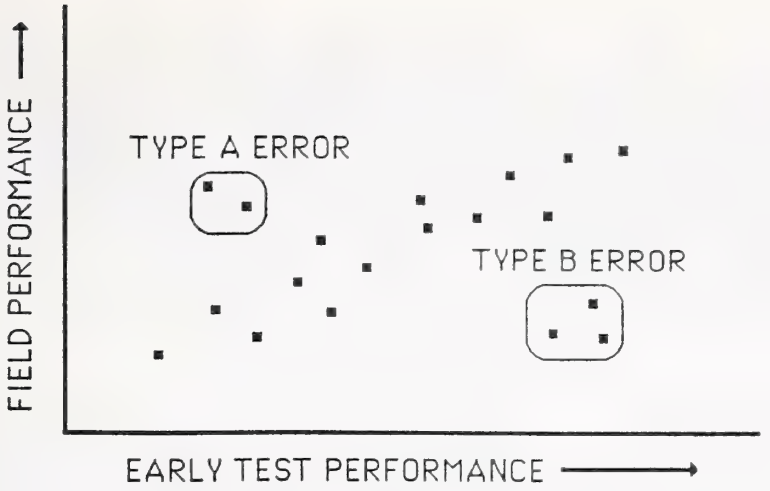

Figure 1. An illustration of type A and type B errors in early testing studies. Type A error involves the rejection of families with good field performance based on early testing data. A type B error occurs when a family with good performance in the early test does not perform well in field tests.

a portion of the families are eliminated prior to the next test. This procedure was first described by Long et al. (1977). Van Buijtenen et al. (1986) described how step-wise testing was applied in a pilot scale breeding project for loblolly pine. It is generally desirable to conduct the least expensive test first on the largest number of seedlings. However, the sequence of tests should consider the genetic relationships among the traits, the precision of the early test, and the relative importance of the individual traits.

Most tree improvement programs have used a single breeding and test design to satisfy the objectives of parental evaluation and advanced generation selection. A complementary system uses a separate mating and field design for each objective. Each design can be more efficient at addressing a specific objective than a single design that addresses both objectives. If the early test for parental evaluation can be completed prior to crossing for development of the selection population, assortative mating can be utilized to increase genetic gains in the selection population. Costs associated with developing the selection population can also be reduced because the rejected families will not be involved in either control-pollination or field testing.

Step-wise testing can be applied to either an open or closed breeding population. Increased genetic gains can be obtained by increasing the selection differential in an open breeding population. If resources restrict field testing activities to 500 families, 665 initial selections could be made if 25 percent of them are rejected on the basis of early testing results. Estimates of genetic gain in this situation would need to consider the effect of indirect selection resulting from the early testing procedure. However, the rejection of poor performing

families prior to field testing will allow the use of allocated resources only on better material. In a closed breeding population, the resources required to conduct the field testing can be reduced by the use of an early testing procedure. The savings in cost reduction should be proportional to the rejection rate from early testing. With a complementary mating system, positive assortative mating can be used to obtain increased genetic gains in either an open or closed breeding population.

IMPLEMENTATION IN THE WGFTIP

The WGFTIP is using step-wise testing to implement early testing procedures in its operational tree improvement programs. Complementary mating designs and positive assortative mating will be used when practical. Because different tests are conducted to evaluate different types of traits, separate early testing procedures have been developed for the three major types of traits.

Selection for Juvenile Trait

Survival and grass-stage emergence are important juvenile traits for longleaf pine (Pinus palustris Mill.) that can be directly evaluated at an early age. Because phenotypic selection is not effective (Goddard et al. 1973) and the large cost involved with long-term tests (20 years), a two-step testing program was selected.

Initially, open-pollinated seed was collected from 470 selections in the Western Gulf Region and established in a series of short-term tests for three years. Each series contained between 70 and 100 families. These tests were established at close spacing on a wide variety of sites. On the basis of two or three year data, families were selected for inclusion in the long-term tests. Families established in the long-term tests had better than average survival and emergence from the grass stage. For the first series of short-term tests, the selected families averaged a seven percent improvement in survival and a six percent improvement in grass stage emergence (Byram et al. 1986). Approximately 125 families out of 470 original selections will be established in long-term field tests.

Juvenile Evaluation of a Mature Trait

Primary emphasis for genetic improvement in the slash pine (Pinus elliottii Engelm.) breeding program of the WGFTIP is to increase resistance to fusiform rust (Cronartium quercuum (Berk.) Miyabe ex Shirai R. sp. fusiforme). The Resistance Screening Center (RSC) at Asheville, North Carolina, has developed techniques to evaluate the rust resistance of selected material at the seedling stage (Anderson

and Powers 1985). This facility can be used as an early testing procedure to improve rust resistance in slash pine.

Open-pollinated seed from 24 slash pine selections belonging to a single breeding group has been submitted for testing at the RSC and established in field trials. To consolidate RSC indexes across different tests, each index was expressed as a standard deviation compared to the susceptible checklot. Any clone that scored less than 0.5 standard deviations above the susceptible checklot was rogued from seed orchards and will be eliminated from the breeding population.

Table 1 presents the results for percent rust infection from the field tests. Rust infection is expressed as standard deviations compared to mean plantation rust infection in progeny tests with at least 30 percent infection and significant effects among genetic entries at the 10 percent level. Families occur in between two and six field tests. A negative score indicates less infection than plantation average. The average rust score for the 24 selections is -0.02. Had the RSC score been used, 10 of the 24 clones would not have been field planted. The average rust score of the remaining 14 selections is -0.32. Even though a type A error (BSS-7) and four type B errors (BSS-14 and BSS-6, K-163 and BSS-9) occurred, use of the RSC would have improved resistance to fusiform rust while reducing field progeny test costs by approximately 40 percent.

Table 1. Standard Field Scores[1] for percent infection by fusiform classified by Resistance Screening Center (RSC) tests. Scores are the percent infection expressed as standard deviations compared to plantation average.

Resistant at RSC		Susceptible at RSC	
Clone Name	Field Score	Clone Name	Field Score
BSS-14	1.65	OIS-3	0.12
K-143	-0.72	OIS-1	0.61
OIS-4	-1.02	K-142	1.03
K-211	-0.48	K-2	0.25
BSS-6	0.88	K-149	0.27
OIS-2	-0.72	MFCS-1	0.00
BSS-10	-1.34	K-141	1.37
BSS-8	-0.03	BSS-7	-1.14
K-179	-0.25	OIS-5	0.79
K-163	0.87	BSS-5	0.73
PE2	-1.60		
BSS-13	-1.08		
BSS-9	1.03		
BSS-11	-1.65		

The WGFTIP uses a partial-diallel for its first generation breeding program (Figure 2). Second generation selections are made from the best 15 percent of the families based on midparent values in field progeny tests. The clones shown in Table 1 were randomly mated in the standard Western Gulf partial-diallel for 50 computer iterations. After each iteration, the best 15 percent (seven) of the full-sib families were selected for rust resistance. Across the 50 iterations, the selected families averaged 1.06 standard deviations less rust infection. To evaluate the effects of both the type A and B errors shown in Table 1 upon second generation selection efficiency, only the 14 parents rated as resistant at the RSC were also randomly mated for 50 computer iterations. The best seven full-sib families selected from each iteration averaged 1.16 standard deviations less rust infection. Assortative mating of the 14 selections rated as resistant at the RSC, resulted in selecting families that averaged 1.07 standard deviations less rust infection. The occurrence of four type B errors in the selected clones was detrimental to the possible benefits of assortative mating.

		MALE								
		A	B	C	D	E	F	G	H	I
	A		X	X						
F	B			X	X					
E	C				X	X				
M	D					X	X			
A	E						X	X		
L	F							X	X	
E	G								X	X
	H	X								X
	I	X	X							

Figure 2. WGFTIP first generation partial-diallel mating scheme.

Rejection of slash pine selections from the breeding population that indicated susceptibility to fusiform rust at the RSC would result in approximately a 40 percent reduction in costs associated with field testing. An opportunity exists to also obtain an increase in genetic gain.

The open-pollinated seed submitted to the RSC was collected at different locations and in different years which resulted in different pollen clouds among the selections. The resistance or susceptibility of the pollen to fusiform rust has been shown to affect family rankings at the RSC (Byram et al. 1987). The use of stable rust susceptible pollen may improve the agreement between RSC and field tests and eliminate a portion of the errors observed among these selections.

Juvenile Trait Correlated with A Mature Trait

Early testing studies with loblolly pine in the Western Gulf region have indicated that shoot dry weight at 6 months was the seedling trait most consistently correlated with field data (Waxler 1980, Miller 1982, Davison 1984 and Strickler 1984). These studies were conducted under greenhouse and/or growth chamber conditions and indicated varying degrees of success in predicting field performance.

A recent review of these and other unpublished studies indicated that type A errors were eliminated when an independent culling level based on germative capacity was used (Figure 3). The deletion of families with less than 85 percent germination eliminated the error of rejecting fast growing families on the basis of shoot dry weight data obtained from greenhouse tests. These studies included approximately 60 different families from different geographic areas within the Western Gulf Region.

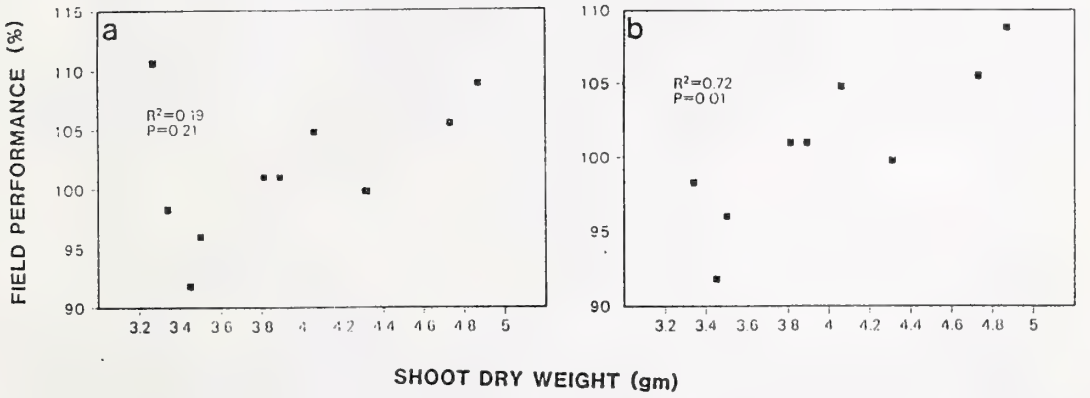

Figure 3. Scatter diagrams, coefficients of determination (R^2) and the occurrence of type A error in Strickler (1984) (a); type A error is eliminated after culling at 85% germinative capacity (b).

Families with small shoot dry weight tend to have average or below average performance in field tests. Step-wise testing can be used to incorporate this early testing procedure into operational breeding programs. Lowe and van Buijtenen (1989) describe how shoot dry weight based on greenhouse data is used in both the first and second generation loblolly pine breeding program of the WGFTIP.

The first-generation breeding program utilizes a partial-diallel mating design (Figure 2) which is planted in three tests. With this design, the crosses containing the parent with the smallest shoot dry weight is not field planted. A composite checklot composed of an equal number of seedlings from each of the rejected families is planted with the test to evaluate the early selection procedure. This results in approximately 17 percent fewer test trees being planted. The replication size is reduced and the costs associated with planting, maintaining and measuring the field test is reduced. Families that were

rejected on shoot dry weight data are not planted in later progeny tests.

A complementary mating design will be used in the second generation breeding program. Parental general combining ability estimates will be obtained from polymix crosses and the partial-diallel shown in Figure 2 will be used to develop a population for the third generation selection activities. Greenhouse shoot dry weight data will be used to reject 30 percent of families prior to planting the polymix field tests. The rejected selections will not be crossed in the partial-diallel mating scheme. Shoot dry weight data and mid-parent values will be used to assortative mate the second generation selections in the partial-diallel. Because pollen from the previous generation is used in the polymix, the length of the breeding cycle should not be increased because of the step-wise testing procedure.

Field testing costs will be reduced because approximately 24 percent fewer test seedlings for the polymix crossing scheme will be established. Additional cost savings will result from making and field planting fewer crosses in the partial-diallel portion of the testing program. An evaluation of six studies indicated that assortative mating using shoot weight data resulted in a 1.2 percent increase in genetic gain in the selected breeding population (Lowe and van Buijtenen 1989).

CONCLUSIONS

Early testing procedures can be applied to operational tree breeding programs to increase efficiency by reducing the costs associated with field testing. Step-wise testing is a useful component of an early testing procedure. After each test, a portion of the families are rejected before the next test. This procedure can be applied to either open or closed breeding populations. Complementary mating schemes can be used to incorporate positive assortative mating to increase genetic gains.

Important juvenile traits, mature traits that can be evaluated at a juvenile age and juvenile traits that are correlated with an important mature trait are candidates for early testing procedures. The WGFTIP is applying early testing procedures using step-wise testing techniques to improve field testing efficiency to all three classes of characteristics.

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THEORETICAL AND APPLIED ASPECTS OF SHORT-TERM PROGENY TESTING IN LOBLOLLY PINE

E. C. Franklin

Abstract.--Patterns of stand development based on trends in genetic and environmental variances were similar to those previously found for several North American conifers grown at conventional spacing. Trials at 33, 50, 67 and 100 cm square spacings all showed similar patterns of change in genetic and environmental variances over time. For practical as well as theoretical reasons, the widest spacings, 67 and 100 cm offered the most promise for applied short-term testing. Sibling correlations for volume index generally peaked and leveled off at 2 to 3 years of age in all spacings with maximum correlations reaching 0.47 in the 100 cm spacing. In practical terms, 8 of 9 top-ranking families in the 100 cm spacing, were from the top 50% of parents ranked on the basis of long-term tests. Volume per tree and volume per unit area had consistently higher sibling correlations than height. In the 100 cm spacing, sibling correlations for height continued to increase through age 10 years while those for volume remained steady. At the 33 cm spacing, sibling correlations dropped sharply as mortality exceeded 75%. Results indicated that at 3 years from planting, short-term, closely spaced tests can provide a very efficient means of culling large base populations for volume growth. This could substantially reduce the cost of testing for other more complex traits such as structural quality and chemical composition of wood.

Keywords: Pinus taeda L., short-term progeny testing, close spacing, genetic variance, heritability.

INTRODUCTION

The concept of interpreting phases of stand development in terms of progressively changing environments for tree growth, and the resulting changes in expression of genetic and environmental variances was generalized by Ford (1975, 1976). Specific examples in western conifers were reported by Namkoong et al. (1972) and by Namkoong and Conkle (1976). These concepts were integrated into a genetic model of stand development by Franklin (1979) for Douglas-fir (Pseudotsuga menziesii [Mirb] Franco) and ponderosa pine (Pinus ponderosa Dougl. ex Laws), and two eastern conifers: loblolly (Pinus taeda L.) and slash pines (Pinus elliotti Engelm.). Short-term progeny testing has been successfully applied for at least 25 years in southern pines (Squillace and Gansel 1968, Franklin and Squillace 1973) and in eucalypts (Franklin 1986). There is no question that when properly applied, it works well. We need to understand more about why it works and thereby make it work better.

In 1978, a study was begun at the USDA Forest Service's Forestry Sciences Laboratory in Charleston, SC to test two hypotheses:

1. That young stands grown at close spacings would display the same patterns of change in genetic and environmental variances as older stands at conventional spacings.

2. That accelerated stand development could be used to increase the precision of short-term progeny testing by carefully monitoring genetic phases of stand development and the corresponding changes in levels of genetic and environmental variances.

METHODS

The experimental design consisted of four adjoining trials grown at 33, 50, 67, and 100 cm square spacings in a small agricultural field near the laboratory. Each trial was a randomized complete block of 4 replications of 35 families, in 16-tree square plots (Table 1).

Table 1. Companies which made seed available from selected clones in their commercial production orchards (after Franklin, 1983)

Company	No. Families	Provenance
Continental Forest Industries	8	GA Piedmont
Union Camp Corporation	8	GA/SC Coastal Plain
Weyerhaeuser Company	14	NC Coastal Plain
Westvaco	5	SC Coastal Plain

Measurements of total height were made each growing month over a five-year period. December, January and February were classified as non-growing months and excluded from the measurement schedule. Measurements in the 11th, 17th and all growing months thereafter included total height and diameter. Diameters were measured first at one inch above the ground (11th month) then at mid-height (17th through 23rd growing months) and at 4 1/2 feet above ground thereafter. Heights and diameters were measured in alternate growing months in the 21st through the 33rd growing months, the 36th through the 42nd growing months, and individually in the 51st, 69th and 88th growing months.

Two indices of volume were used. Volume per tree was indexed as diameter squared times total height, and that quantity was divided by original growing space to index yield per unit area. For the yield index, zero yield was recorded for dead trees so mortality did not reduce the number of trees in the model, but did reduce the average yield.

Cumulative mortality, total phenotypic variance, additive genetic variance, environmental variance, and heritability were estimated for each measurement. Variances were interpreted based on the simplest additive model:

$$P = A + E, \text{ and } h^2 = \frac{A}{P},$$

where:

P is phenotypic variance,
A is additive genetic variance,
E is environmental variance, and
h^2 is individual narrow-sense heritability (Franklin 1983).

Height and volume family performance levels for conventional progeny trials were supplied by the NC State University-Industry Tree Improvement Cooperative. These arrays were compared to the corresponding family means for height, volume and yield indices from the short-term tests using product-moment correlations.

RESULTS

Mortality

Cumulative mortality did not begin to reflect large differences in growing space until the fourth and fifth years of the study. In 1981, the amount of mortality sharply increased in the 33 cm spacing, and in 1982 in the other spacings (Franklin 1983). Rate of mortality through time was inversely proportional to original area per tree, but by the 88th growing month (10th year) the three closest spacings had achieved about the same average space per tree, while the 100 cm spacing had more growing space but was still showing a high rate of mortality (Table 2).

Table 2. Trends in mortality and subsequent average spacing after 69 and 88 growing months from planting

Original square spacing cm	Original area per tree m^2	Mortality at 69 months %	Mortality at 88 months %	Area per tree at 69 months m^2	Area per tree at 88 months m^2
33	.111	91	95	1.25	2.09
50	.250	76	86	1.05	1.79
67	.458	63	78	1.20	2.01
100	1.000	44	63	1.80	2.70

Patterns of Genetic Variance and Heritability

In terms of the model proposed by Franklin (1979), the juvenile and mature genotypic phases were readily observable (Table 3). The transition into the codominance-suppression phase was apparent for height in the three wider spacings (Figure 1), but was not observed at the 33 cm spacing. Neithervolume nor yield indices had reached the codominance-suppression phase through 88 growing months (Figure 2).

Additive genetic variances for height showed a generally rising trend through the 69th growing month at the 50, 67 and 100 cm spacings. At the 88th growing month, additive genetic variance had decreased at the three wider spacings. This accelerated the already decreasing trend in heritability, which had been decreasing steadily since the mature genetic culmination (MGC). In the 33 cm spacing, additive genetic variance for height and volume

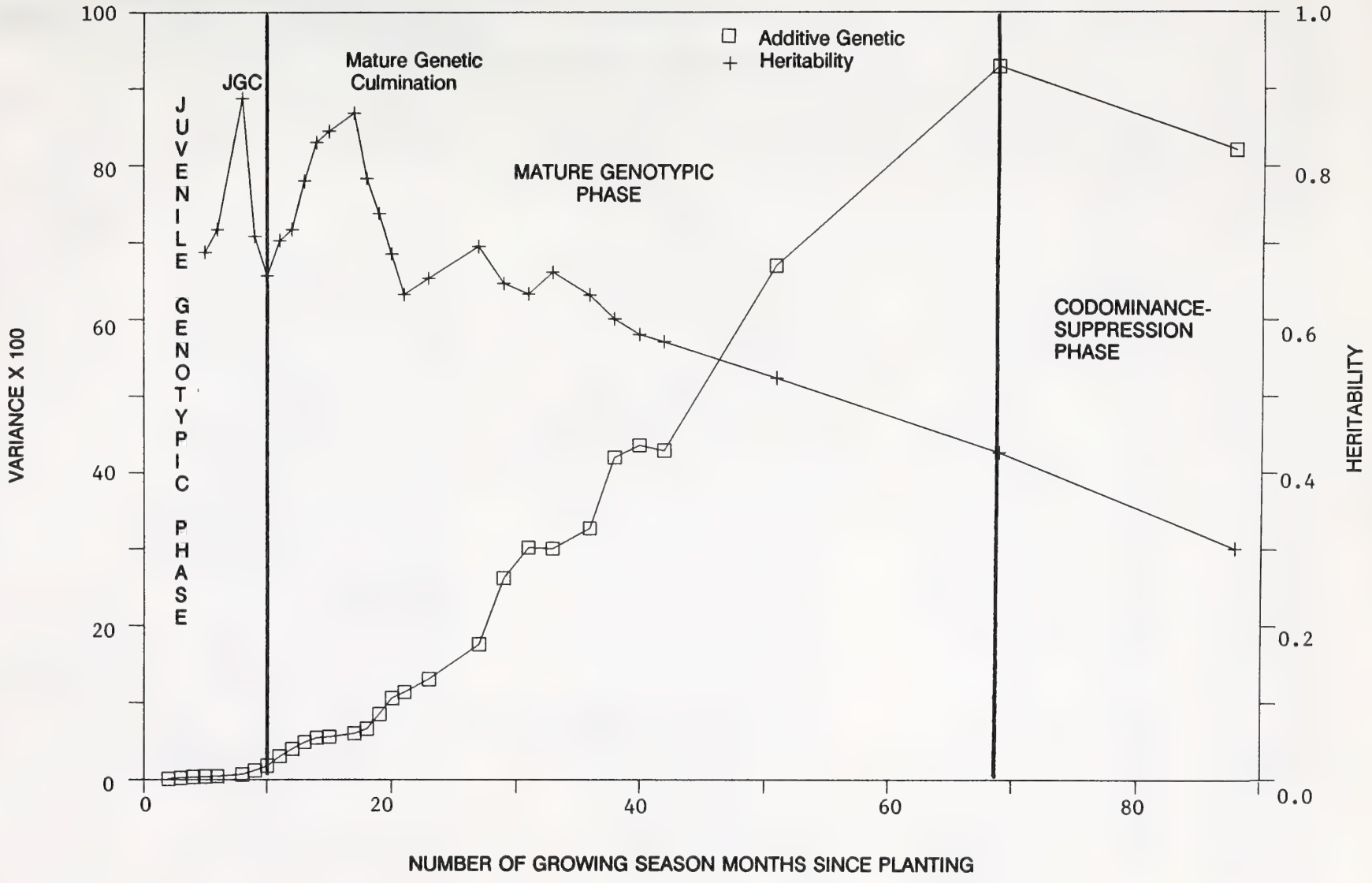

Figure 1. Patterns of genetic and environmental variances for height in the 50 cm spacing, interpreted according to the model by Franklin (1979). Growing month 2: July, 1978; growing month 88: March, 1988.

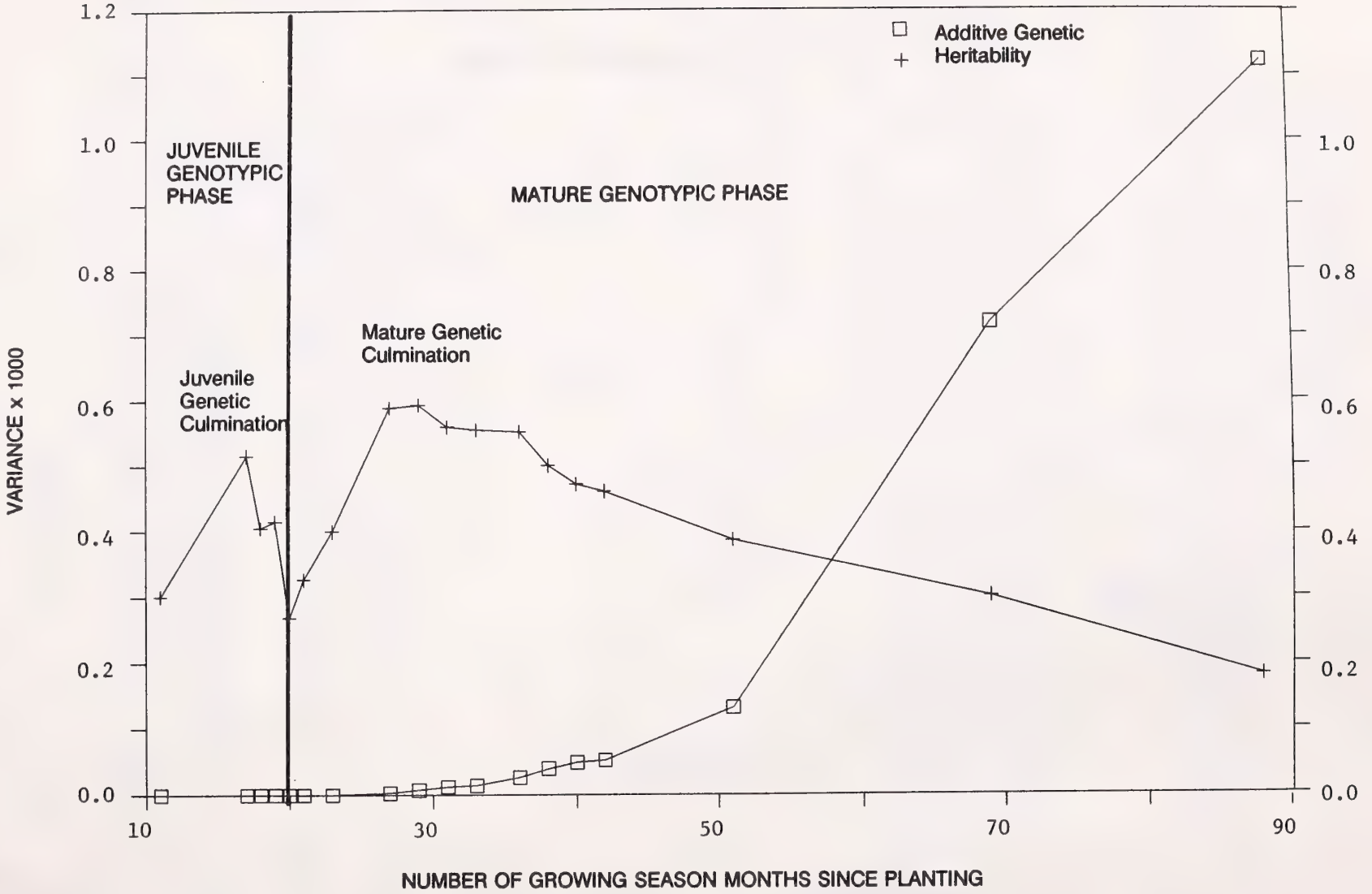

Figure 2. Patterns of genetic and environmental variances for volume per tree in the 100 cm spacing, interpreted according to the model by Franklin (1979). Growing month 11: July, 1979; growing month 88: March, 1988.

continued to increase, and at the 69th growing month, heritability reversed its downward trend and started to increase. These results are probably spurious since by the 69th month only about 200 (9%) of the original trees were alive. This reversal in trend of heritability in the 33 cm spacing did not occur with the yield index, which was analyzed with the full model.

Additive genetic variances for volume and yield indices generally increased throughout the course of the study in all four spacing trials. In contrast, heritabilities decreased at relatively steady rates from the MGC through the 88th growing month.

Heritabilities for height at the MGC ranged from 0.62 to 0.89 over the four spacings, with the intermediate spacings having the higher values. Heritabilities for volume index ranged from 0.36 to 0.63 with the 33 cm spacing having by far the lowest value. Heritabilities for yield index ranged from 0.29 to 0.59 with the 33 cm spacing again having the lowest value (Table 3).

Correlations with Conventional Tests

For height, sibling correlations with performance levels in conventional tests were near zero during the first few months, then gradually increased through about 40 months. Correlations then decreased markedly through 88 months at the three closer spacings. In the 100 cm spacing, correlations continued to increase through 88 months. Patterns of change in correlations for both volume and yield indices were similar. Correlations increased rapidly from 11 to 18 growing months, then leveled off in the 50 and 100 cm spacings, gradually decreased in the 33 cm spacing, and gradually increased in the 67 cm spacing. Yield index tended to decrease less rapidly than volume index. In almost all cases, the MGC was a stage where correlations were at or near their highest levels for volume and yield indices (Table 3).

DISCUSSION

The Model

Patterns of genetic and environmental variances and heritability for height, volume and yield through 88 growing months (10 years) were very similar to those previously reported for loblolly pine through 25 years and Douglas-fir through 53 years. The similarities between the four spacing trials in this study, and between this study and previous studies suggests that a relatively consistent pattern of changes in genetic and environmental variances does occur, and that planting at closer spacings can shorten the time required to see this pattern develop. However, the range of spacings used in this study did not result in any appreciable differences in patterns of development except in rate of mortality. Most mortality, even at the closest spacing (33 cm), occurred well after the MGC for volume and yield indices (27-29 months).

Table 3. Number of growing months to reach successive stages in a stand development model (Franklin 1979), and corresponding heritabilities and correlations with results of older trials

Trait	Spacing	Juvenile genetic culmination (mo)	Juvenile mature phase change (mo)	Mature genetic culmination (mo)	Codominance-suppression phase change (mo)	Individual Narrow-Sense Heritability at the MGC[1] h^2	Correlation at MGC[1] for volume r	Highest correlation at any age r
Height	33	8	13	17	NO[2]	.72	.32	.37
	50	8	10	17	69	.87	.32	.39
	67	8	10	17	69	.89	.19	.27
	100	8	12	17/36[3]	69	.62	.25	.38
Volume per Tree								
	33	18	21	23	NO	.36	.38	.42
	50	18	20	27	NO	.50	.32	.32
	67	17	23	27	NO	.60	.26	.36
	100	17	20	29	NO	.63	.47	.48
Yield (Volume per Unit Area)								
	33	18	21	27	NO	.29	.42	.42
	50	18	20	27	NO	.49	.32	.32
	67	17	23	27	NO	.59	.36	.36
	100	17	20	27	NO	.48	.44	.47

[1]Mature genetic culmination.
[2]Not observed through 88 growing months.
[3]In the 100-cm spacing, a sharp, distinct peak occurred at 17 months, but a rounded less distinct peak which was 0.05 units higher occurred at 36 months.

This study has shown that accelerated stand development can be obtained by planting loblolly pine at square spacings of from 33 to 100 cm. It has failed to show any shortening of the cycle of development due to reduction of spacing within the range tested.

Operational Testing

With regard to operational use of short-term progeny testing, results of this study indicated that over the range of spacings tested, the same results will be obtained in rate of change in genetic structure. Other criteria of importance might include statistical stability, size of heritability at the MGC, strength of correlations with conventional tests, efficient use of space and ease of measurement (Table 3). The 33 cm spacing proved to be the poorest with regard to these criteria. It was difficult to measure, yielded low heritabilities, especially for volume, and by the end of 10 years most (95%) of its trees were dead. This precludes the use of the study for other traits such as wood quality. The 50 cm spacing was marginally to substantially better but still not as good as the 67 and 100 cm trials.

The best trait to use as indicated from these results is either volume per tree or yield (volume per unit area). If mortality is a factor to be considered, volume per unit area is the preferred trait. In this study, mortality was very light at the MGC, so both traits behaved similarly. Volume per tree had slightly higher heritabilities than yield at MGC, and equally high correlations with long term tests.

There were two criteria examined in this study to determine the optimum time to measure a short-term test: the highest heritability (MGC) during the mature genotypic phase, and the time when the correlation with long-term conventional tests were highest. Results of the study indicated 27 to 29 growing months or about 3 years from planting as the optimum age at which to measure. This was the point of maximum heritability (MGC) and also a time when correlations with long-term tests were at or near their highest values for volume and yield indices. In practical terms, a correlation of 0.47 for volume per tree in the 100 cm spacing meant that of 9 families in the top 25% in the short-term test, 8 were in the top 50% of the long-term test. Thus, the correlations are high enough to prove the value of short-term tests, however we would like to see even higher correlations in future tests. Higher correlations might have been obtained if only local families had been used. Families from Westvaco's orchard consistently had extremely high correlations (0.90+) with older tests for height, volume and yield indices. Families from a piedmont source showed much lower correlations. Use of control-rather than orchard open-pollinated material would be expected to strengthen correlations because each orchard has a unique pollen background. Use of genetically proven check families would also be expected to improve precision in short-term tests (Franklin and Squillace 1973).

CONCLUSIONS

Short-term progeny tests in this study reduced the optimum time of measurement for height in loblolly pine from 10 years to 2 years and for volume from 20 years to 3 years based on results by Franklin (1979). Patterns

of genetic and environmental variances and heritability through time in the short-term tests were similar to those previously found in long-term tests, but in a much compressed time-frame. A ten-fold difference in growing space per tree did not affect the optimum time for measurement in this study. Other considerations indicated that the 67 and 100 cm spacings were preferable over closer spacings. Correlations of short-term and long-term test results were high enough to be useful, but could probably be improved by use of local seed sources, control-crossed material, and genetically proven checks.

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DETERMINATION OF OPTIMUM SELECTION AGES: A SIMULATION APPROACH

S. Magnussen[1]

ABSTRACT. Prediction models of growth (height and stem volume), phenotypic variation, heritability, and age-to-age correlations provided an integrated framework for predicting optimum selection ages in a breeding program of fixed duration. The risks of early selection were assessed by computing a minimum significant gain ratio of juvenile selection; only gain ratios exceeding this minimum were permissible options for juvenile selections. Optimum selection ages were either very low (usually below one-sixth of the rotation age) or equal to the rotation age itself. Although intermediate selection ages promised more gain per unit time than selection at rotation age, the gain did not outweigh the associated risk. Slow growth (high rotation) made juvenile selection more attractive than it was in the fast growth scenarios. Minor changes in model parameters often had dramatic impacts on the age of optimum gain ratio. The simulation approach to estimation of optimum selection ages provides an useful tool for sensitivity analyses of a gain expression with many interrelated parameters.

Keywords: Early selection, simulation, decicion making, heritability, correlation, genetic gain

INTRODUCTION

Determining the selection age that achieves the highest rate of genetic progress in a tree improvement program requires information on time trends in variances, heritabilities, and age-to-age correlations (Falconer 1981, Lambeth 1980). Sufficient information is rarely available; so the choice of selection age is frequently based on general prediction models (Lambeth 1980), the strength of age-to-age correlations in existing trials (Cotterill and Dean 1988, Foster 1986, Lambeth et al 1983, Magnussen 1988, Riemenschneider 1988, Williams 1987), or eclectic experience. The potential pitfalls of parsimonious

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information is widely recognized (Franklin 1979, Kang 1985, Namkoong et al 1972, Nienstaedt 1984, Rehfeldt 1983).

Simulation of competing hypothetical time-trends within parameters of the genetic gain expression provides a simple tool for sensitivity analysis; it also helps bracket the range of the most likely optimum selection age. Accepted solutions should be robust against moderate changes in the parameters of genetic gain.

The present study illustrates how simulation can assist the breeder in deciding selection age. Within a fixed time horizon, different scenarios of growth, variances, heritability, and age-to-age correlation are formalized mathematically and the optimum selection age found by computing the predicted gain per unit time for all possible outcomes. The risk of early selection is considered by requiring that the prospective reward of early selection outweighs the potential risk.

MODEL PARAMETERS

Under consideration is mass selection for tree height or stem volume in a progeny trial. Genetic gains are attained at harvest of improved seed whereas commercial gain is realized by harvest of stands grown from improved stock. Improvement in economically mature traits is, therefore, the objective of breeding efforts. The expected gain (G) in a mature trait (m) arising from selection on trait (j) is equal to:

[1] $G_{m:j} = i*h_j*h_m*r_{jm}*\sigma_m$

(Falconer 1981)

where i = selection intensity
h_j, h_m = square root of heritability
r_{jm} = correlation between j and m
σ_m = phenotypic standard deviation of trait m

Henceforth j and m signifies the juvenile and mature expression of the same trait at time T_j and time T_m, repectively.

In a continuous breeding program the cumulated total genetic gain produced per year (CG) in a given total time (TT) equals:

$$[2]\ CG_{m:j} = \sum_{q=1}^{C-1}\{\sum_{n=1}^{N_c}(q*G^c_{m:j})/((q*T_j+d)+s*(n-1))+ \sum_{n=0}^{N_F-1} c*G_{m:j}/(c*(T_j+d)+s*n)\}$$

where

c = number of breeding cycles in a given total time TT,

$c = TT/(T_j+d)$

d = delay between selection and production of improved seed

s = years between seed harvests

N_C = number of seed harvests during a breeding cycle

$N_C = (T_j+d)/s$

N_F = number of seed harvests after the last breeding cycle

Default values for TT, d, and s were 150, 5, and 3 , respectively.

In order to calculate $CG_{m,j}$ we must know the time trends in heritabilities (h^2), variances (σ^2), and correlations (r). Models of heritability are depicted in Figure 1. All fourteen models have an average heritability of 0.20 in the interval from one to 40 years, a realistic expectation (Foster 1986, Zobel and Talbert 1984).

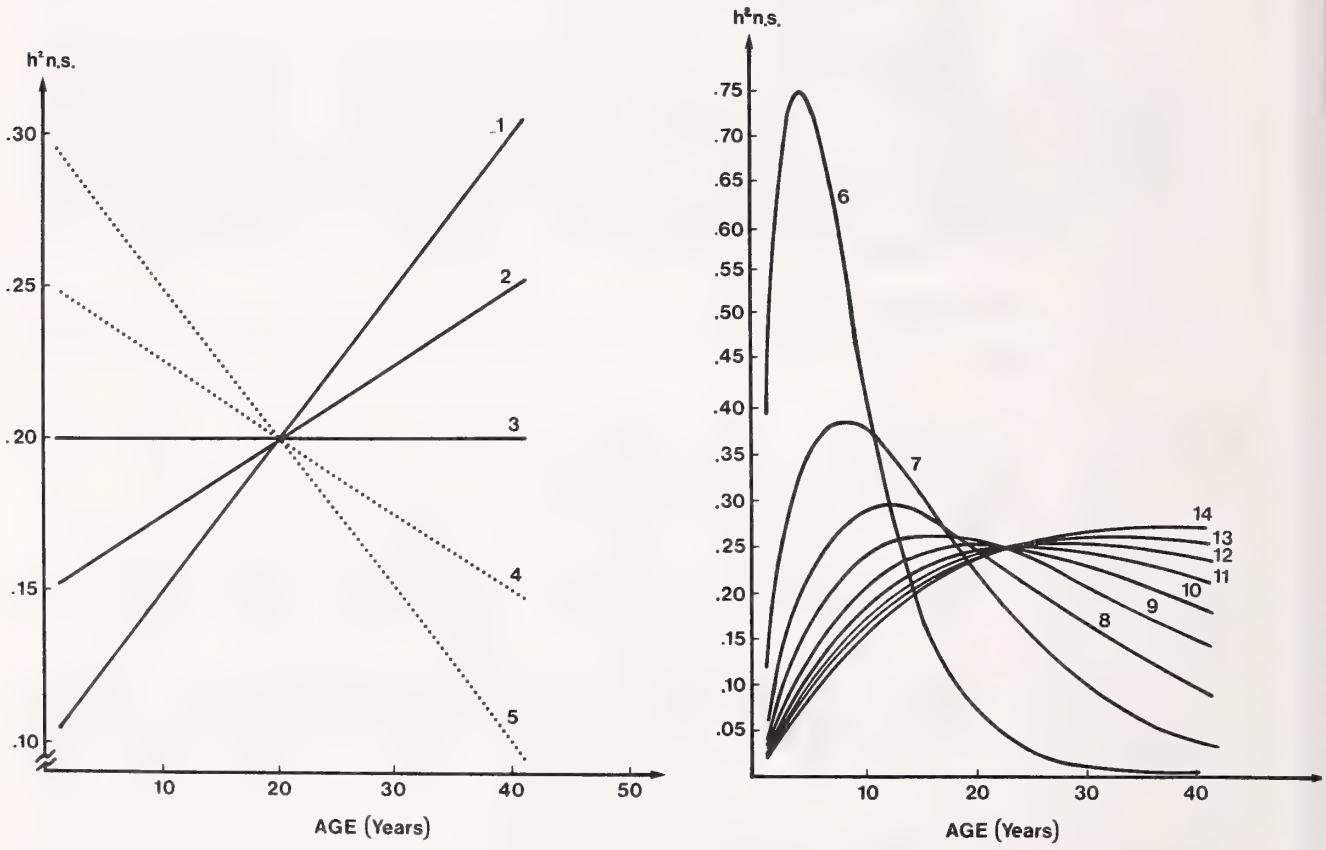


Figure 1. Fourteen models of age trends in heritability.

Time trends in variances were derived by combining the models of tree growth (height and stem volume) in Figure 2 with the trend models of coefficient of variation in Figure 3. It is assumed that actual growth figures will fall within the outlined limits.

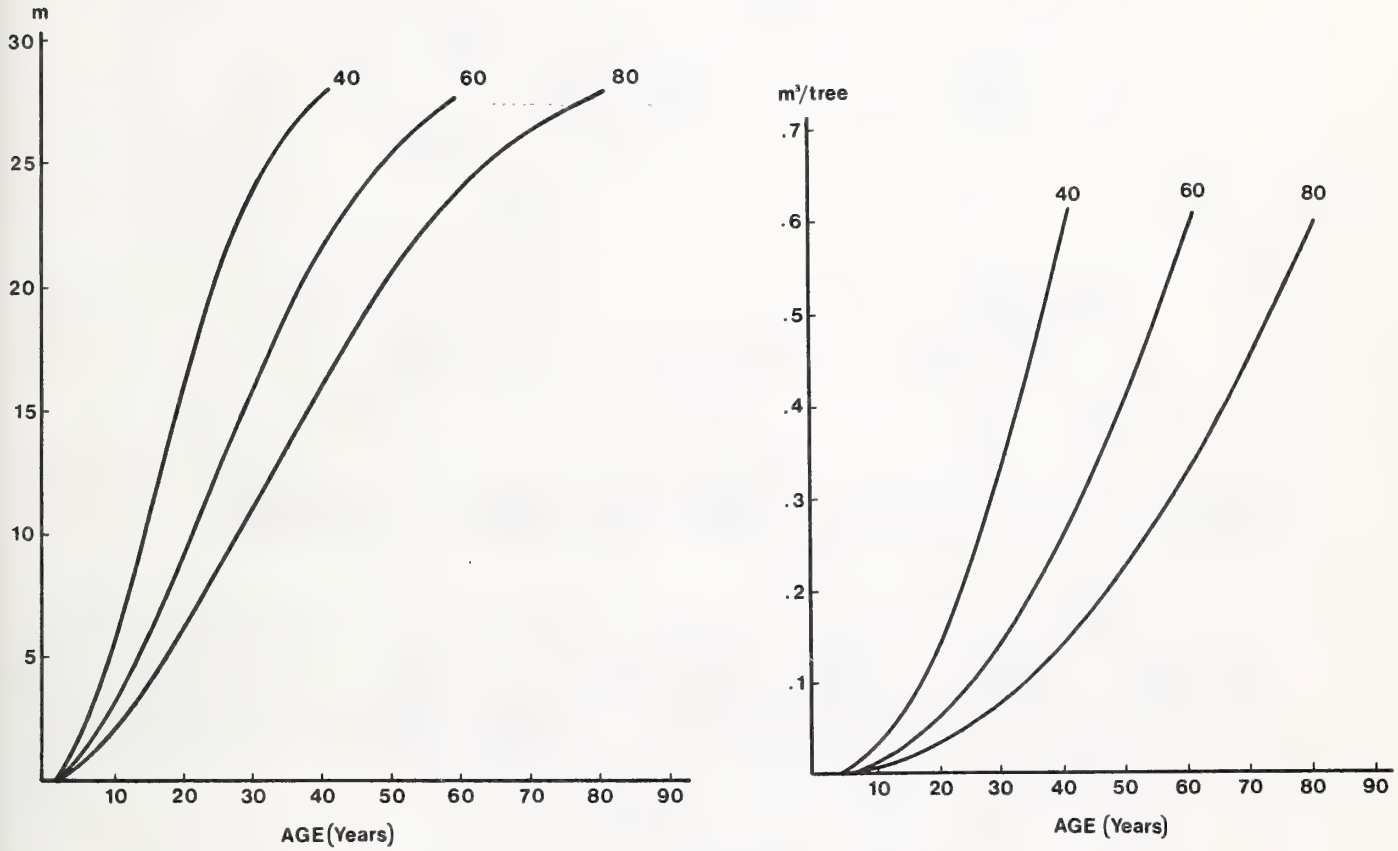


Figure 2. Height (left) and stem volume (right) over age. Rotation length in years is indicated at each curve.

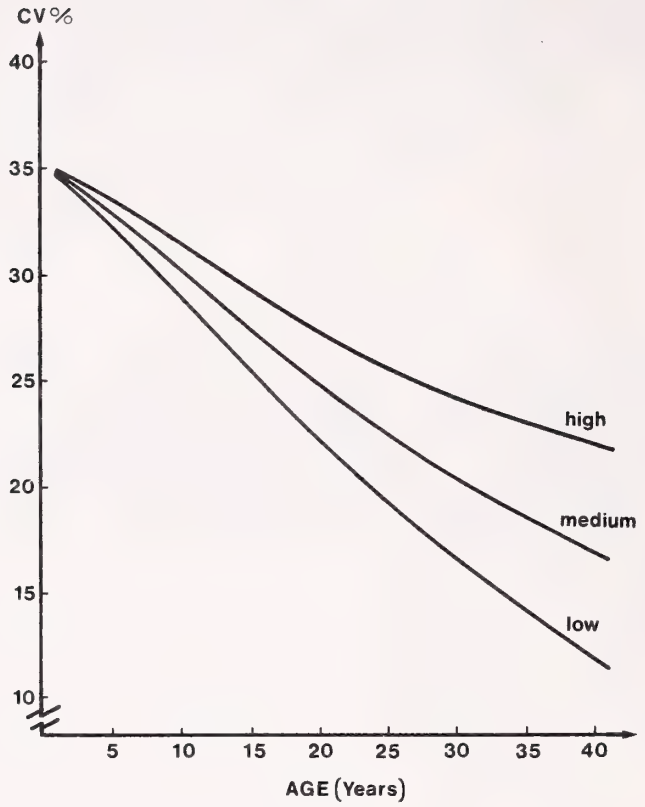
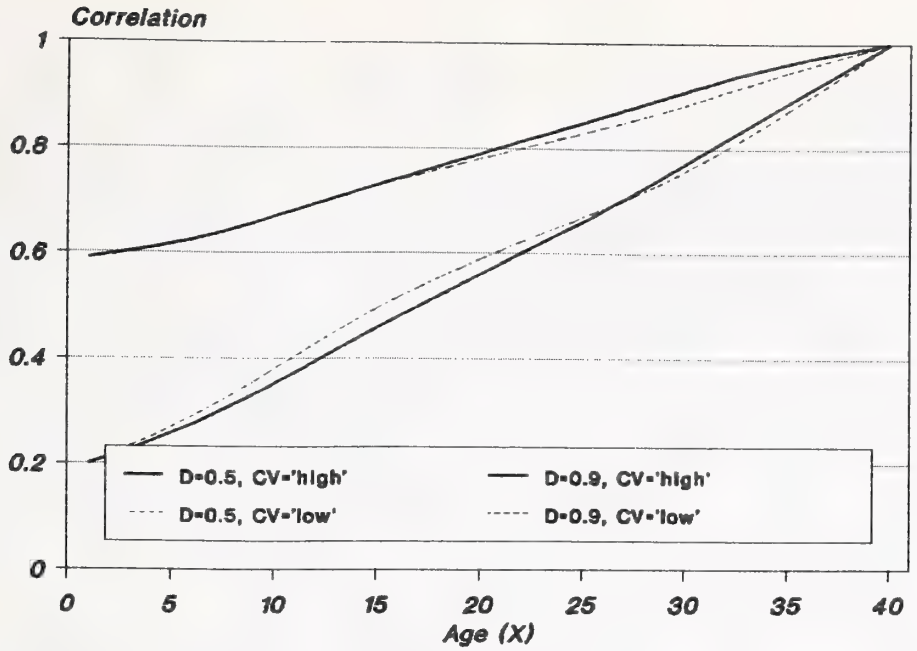


Figure 3. Time trends in the coefficient of variation of tree height and stem volume.

Correlations of trait values at time T_j and time T_m were computed from the ratio of the associated variances ($VR = \sigma^2(T_j)/\sigma^2(T_m)$), the fraction (D) of size dependent growth ($0<D<1$) and an intrinsic growth rate (k_{jm}) during time $T_m - T_j$. More specifically:

[3] $r = (1+k_{jm}*D)*\sqrt{(VR)}$ (Magnussen 1989)

D-values were selected between 0.5 and 0.9 in steps of 0.1, which should bracket realistic expectations (Magnussen 1989). The intrinsic growth rate is obtained indirectly from growth models. Figure 4 illustrates the significance of D and the level of variability ('high', 'medium', or 'low') on the expected age-to-age correlations of height and stem volume.



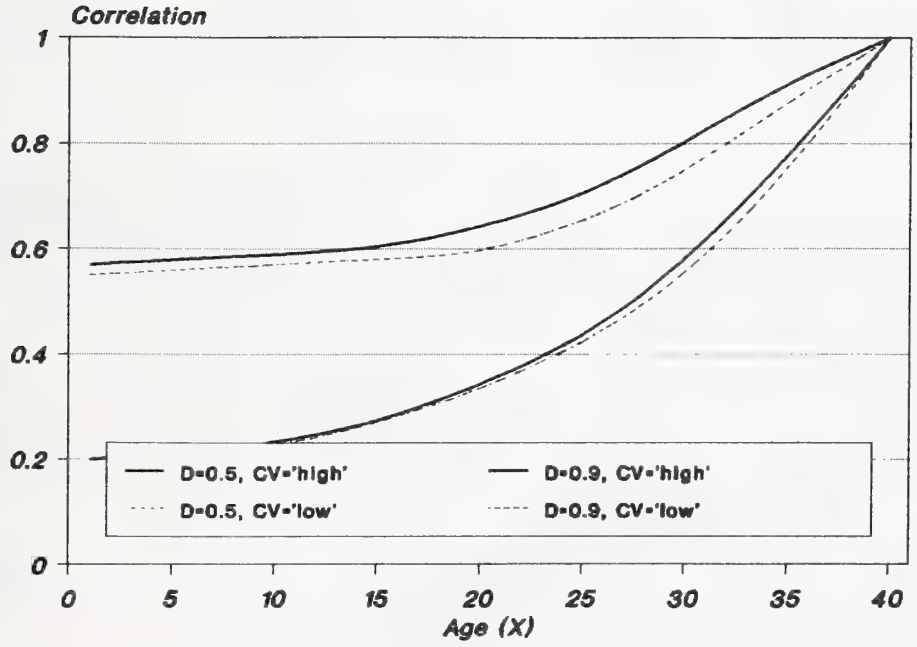


Figure 4. Examples of age 'x' to age 40 correlations. Top: Tree height. Bottom: Stem volume.

Optimum selection age is the age (T_j) that, for a given rotation age (T_m), maximizes the gain ratio $CG_{m:j}/CG_{m:m}$. The standard error of this ratio is cumbersome to estimate (Magnussen 1987). Of the many variables that influence error none is more important than the error of heritabilities (Hallauer and Miranda 1981, Magnussen and Yeatman 1987, Namkoong 1979). Let the standard error of a heritability estimate be 40% (Cotterill and Dean 1988, Dean et al 1983, Foster 1986, Loo et al 1984, Magnussen and Yeatman 1987), the correlation between h_j and h_m be 0.7, and ignore the errors of age-to-age correlations and of the phenotypic standard deviation. Thus simplified we obtain the following expression for the relative error of the gain ratio of juvenile to mature gain per unit time (Magnussen and Yeatman 1987, page 59):

[4] $CV_{\text{gain ratio}} = \sqrt{0.2960*(1-r_{jm})}$

Selection before rotation age is deemed attractive whenever the predicted gain ratio exceeds 1.0 by a significant margin. By accepting a 10% risk of choosing a selection age that actually will yield less gain per unit time than selection at maturity, the decision rule becomes one of finding the maximum gain ratio that, under assumed normality, satisfies:

[5] $CG_{j:m}/CG_{m:m} > 1/(1-1.29*CV_{\text{gain ratio}})$

An example of time trends in the minimum acceptable gain ratio and in the expected gain ratios is provided in Figure 5. Permissible selection ages fall within the cross-hatched area between the two curves.

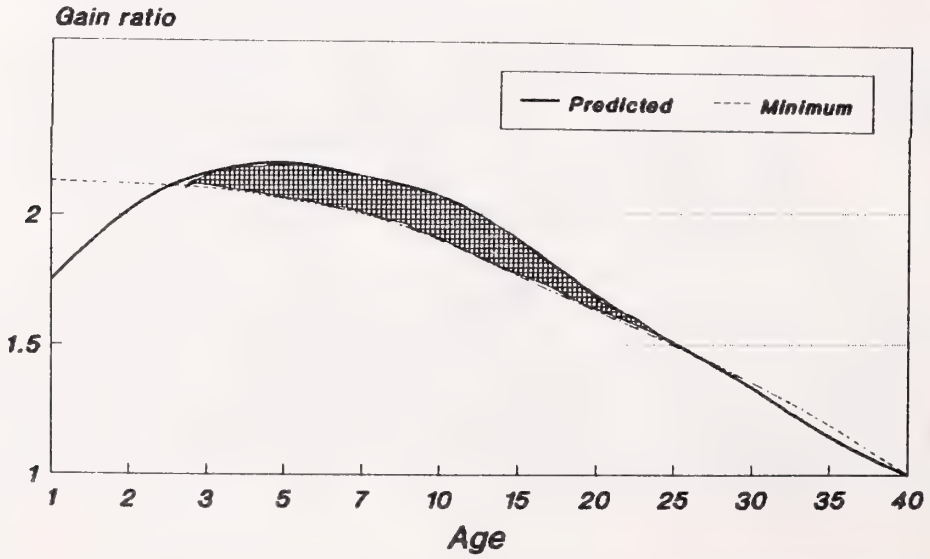


Figure 5. Predicted and minimum significant gain ratio plotted against age. Model: Rotation=40 years, D=0.6, CV%='high', and h2=curve no. 12.

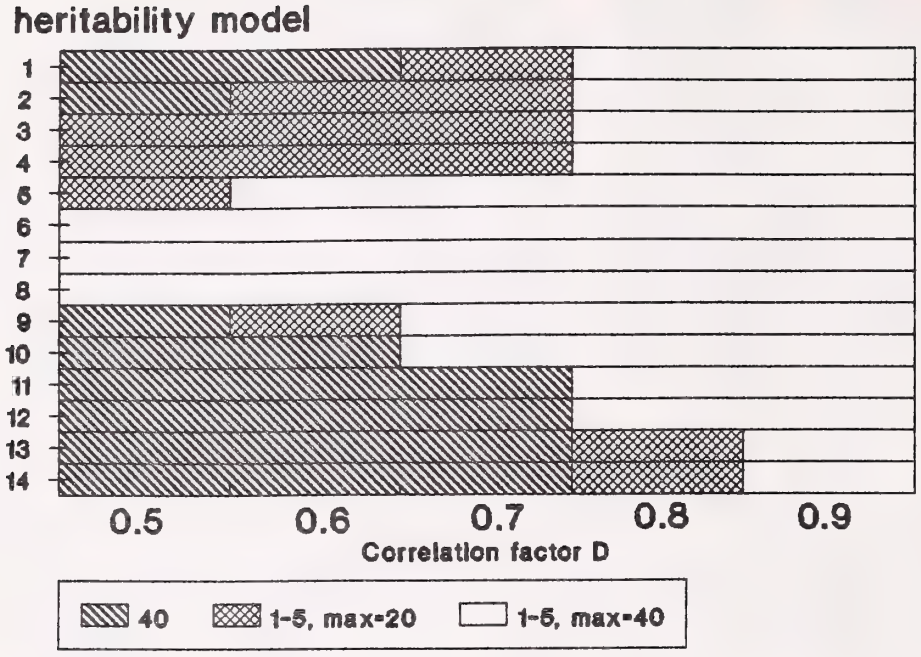
RESULTS

Optimum selection age were either equal to rotation age (T_m) or below five years. A schematic presentation of the most promising selection age for the various combinations of heritability model and level of age-to-age correlations (D-factor) is given in Figure 6 for a rotation age of 40.

It is clear that the combination of low age-to-age correlations (D<0.7) and a heritability rising with age or showing a late culmination (as in models 1,2,10,...,14) leads to postponements of selections until rotation age has been reached. Conversely, the expectation of a falling heritability (model 4 and 5), perhaps after a brief juvenile peak (model 6,7,and 8), justifies selections before age 5. Finally, high age-to-age correlations (D>0.7) support a selection before age 5, regardless of heritability. Such early selections hold the promise of two to 20 times as much gain per unit time (average is about seven times) as selections at rotation age. A delay of five to 10 years beyond the optimum selection age can still be expected to produce about twice the amount of gain per unit time as selection at maturity. However, selections should not be postponed unnecessarily because the involved risk quickly outstrips the diminished reward. This is illustrated in Figure 6 where an upper limit of 20 years is imposed on several solutions. The limit signifies that the additional gain per unit time arising from selection between age 20 and rotation is marginal compared to the associated risk.

Solutions were somewhat trait dependent. The juvenile-mature correlations predicted for stem volume were for any given age and D-value lower than the corresponding height correlations (Fig. 4) that raised the optimum selection age for stem volume, especially in models with a decline or a late culmination in heritability (Fig. 6). Also, the ability to select with some advantage between age 1 and rotation age is much more restricted for stem volume than for tree height. Most early selections of stem volume have to be completed in the first half of the rotation.

Optimum selection ages for a rotation age of 80 were, as a rule, well below the corresponding 40-year rotation results. Only the combination of low age-to-age correlations (D=0.5) and rising heritabilities (models 1,2,11,..,14) conditioned selection ages to fall within five years of the rotation age. The advantage of early selection was much higher for this long rotation; when carried out before age five the gain per unit time increased by a factor between 3 and 50. Results for a rotation of 60 years are intermediate to those outlined above.



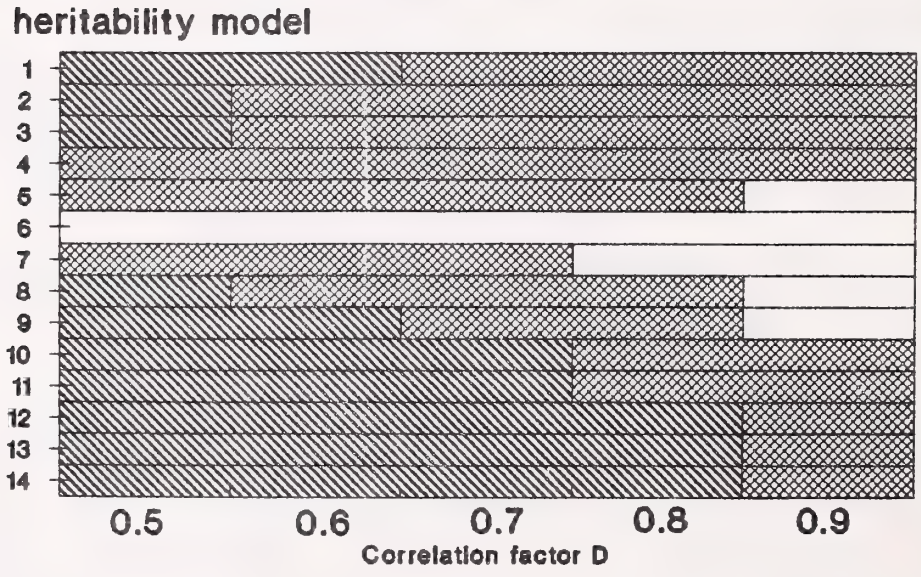


Figure 6. Schematic of optimum selection ages for various combinations of heritability model and correlation factor (D). Maximum age indicates the upper limit of selections with a gain ratio exceeding the significance requirements. Top: Tree height. Bottom: Stem volume.

A factor of little or no influence was the coefficient of variation. The neglible impact of this parameter on the correlations is illustrated in Figure 4a,b. Optimum selection ages for scenarios that only differed in the level of coefficient of variation were less than one year apart.

It is, of course, of little practical value to consider simultaneously the solutions to all the models included in this study. In practice only a few likely alternatives are enumerated and the optimum selection age is taken as the average solution. Examples are provided in Table 1, where four contrasting choices (A,B,C, and D) of the expected time trends in heritabilities and correlations have been identified along with their average optimum selection ages. Scenario A describes a situation in which heritabilities are expected to increase with time or show a late culmination and where correlations are expected to be below average. Scenario B is identical to A with regard to heritabilities, but correlations are assumed to be strong. The last two scenarios, C and D, reverse the heritability expectations in A and B, respectively.

Solutions for scenarios C and D were identical regardless of trait, rotation age, and the strength of age-to-age correlations. For C and D selection at age two is apparently the optimum strategy under conditions covered by the underlying model assumptions (Table 1). Juvenile selections are not recommended for scenario A unless selection is for height and rotation is about 80 years. Scenario B, which differs from A only with regard to the expected age-to-age correlations, permits optimum selection between ages four (height, rotation=80 years) and 17 (stem volume, rotation=40 years). Optimum solutions for stem volume were as a rule higher than for tree height, and higher for short rotations (40 years) than for longer rotations (80 years).

DISCUSSION

The main purpose of this study was to introduce the simulationapproach to determine selection age; realistic values of the parameters in the gain equation are desirable, but not necessary. Simulation is attractive because it brackets unforseeable fluctations in the actual parameters caused by transient technical factors, delayed trait expressions, environmental changes, or ontogenetic phases (Bongarten and Hanover 1985, Dietrichson and Kierulf 1982, Nanson 1969, Nienstaedt 1984, Roulund et al 1986, Skröppa and Dietrichson 1986, Ununger et al 1988, Williams 1988). To limit the scope of this study only single stage selections for a single trait were modelled, with several parameters treated as constants instead of variables. Added realism

Table 1. Optimum selection ages for tree height and stem volume in four hypothetical scenarios of expected time trends in heritabilities and age-to-age correlations.

Scenario	Heritability models	Correlations, D=
A	1,2,11,12,13,14	0.5,0.6,0.7
B	1,2,11,12,13,14	0.7,0.8,0.9
C	4,5,6,7	0.5,0.6,0.7
D	4,5,6,7	0.7,0.8,0.9

Scenario	Height (σ)	Volume (σ)
	Rotation = 40 years	
A	34 (3)	34 (4)
B	12 (4)	17 (5)
C	2 (1)	2 (1)
D	2 (1)	2 (1)
	Rotation = 80 years	
A	9 (4)	24 (8)
B	4 (1)	5 (1)
C	2 (1)	2 (1)
D	2 (1)	2 (1)

could have been achieved by stochastic simulation (Harris 1964, Karlin and Taylor 1975, Ripley 1987, Sales and Hill 1976) or by implementing Bayesian forecasting principles (Harvey 1984, Magnussen 1988). Techniques to enhance gain by multistage selections or by index selections have been dealt with elsewhere (Baradat 1976, Bridgwater and Williams 1987, Cotterill and Dean 1988, Hühn 1987, Magnussen 1987, Young 1964).

Integration of the age-to-age correlation model with models of growth and variability (Magnussen 1989) furnished a better framework for simulations than correlation models with time as the only independent variable (Lambeth 1980). Rotation age was treated as a variable within fixed time limits on the breeding activities and not as a variable that determines the duration of a breeding program (Lambeth 1980, Kang 1985, Porterfield et al 1975, Squillace et al 1967). This distinction is important because the optimum selection age actually decreases with increasing rotation age when the overall time is fixed, whereas it increases with rotation age when no distinction is made between rotation age and total breeding time.

Quantification of risk was another important aspect of the simulation approach. Although risks of juvenile selections are widely recognized (Dietrichson 1964, Loehle and Namkoong 1987, Nanson 1969, Rehfeldt 1983, Steinhoff 1974, Wakeley 1971), few attempts have been made to quantify and incorporate such risks in the context of finding the optimum selection age (Kang 1985, Namkoong et al 1972, Robinson and van Buijtenen 1979,). Time trends in risk and in genetic gain were frequently related in a way that promoted either early selections at a time when the expected benefits outweighed the risk or at rotation age when the recognized risk was at a minimum. Without the risk virtually all selection ages would have been fallen in the interval between one and five years.

The level of variability exercised little or no influence on the optimum selection age. This somewhat surprising result was due to the particular formulation of the correlation model, in which variances and growth rates were determining parameters. A time dependent correlation model would have isolated the significance of the coefficient of variation more explicitly.

A high sensitivity of selection ages to parameters in the gain equations was amply demonstrated. Minor changes in either the correlation function or the heritability model resulted occasionally in a dramatic shift in the optimum selection age. This indicates very flat vertices in several of the analysed models. Also, the effect of heritability depends on the strength of correlations and vice versa, which confirmed results published by Kang (1985). Optimum

selection ages should, therefore, not be interpretated in a narrow sense as an unique solution. Rather, an age interval of 4 to 6 years centred around the optimum age should guide the breeder.

Economic aspects of optimizing the selection age were ignored in this study. High costs and a high discount factor will create a downward push on the results reported herein (Magnussen 1987). A total assessment of all aspect of a breeding program (Cornelius and Morgenstern 1986, McKeand and Bridgwater 1986, Porterfield et al 1975, Thomson et al 1987), including selection age is, of course, preferable but beyond the scope of this paper.

It has been implicitly assumed that progeny testing and subsequent deployment of improved stock took place within a single environment. Genotype by environment interactions will lower age-to-age correlations across sites and a minimum time may be required for those interactions to develop (Bongarten and Hanover 1985, Dietrichson 1964, Rehfeldt 1983). Higher selection ages must be anticipated in programs with substantial genotype by environment interactions within a single breeding zone.

The prevalent numerical superiority of early selection confirmed the overriding importance of time in determining the rate of genetic improvement. Low age-to-age correlations or adverse trends in heritabilities did little to detract from the benefits of early selections. Only the associated risk called for a significant delay in the optimum selection age. Similar results have surfaced regularly in the literature (Franklin and Squillace 1973, Gill 1987, Hatchell et al 1972, LaFarge 1972, Lambeth et al 1983, Loo et al 1984, Robinson and van Buijtenen 1979, Squillace et al 1967, Squillace and Gansel 1974, Wakeley 1971, Ying and Morgenstern 1979).

CONCLUSIONS

Simulation provides a valuable and easy to use tool for the complex problem of finding an optimum selection age in forest tree breeding programs. A breakdown of the equation of genetic gain into single parameter models enables the breeder to enumerate all possible outcomes of the process at hand. Combined with the risk profile of the organization this simulation excercise will help identify the window of opportunity and limits of early selections.

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SYCAMORE SEED AND SEEDLING TRAITS--
EARLY SELECTION CRITERIA?

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Abstract.--American Sycamore (Platanus occidentalis L.) is a candidate species for short-rotation energy plantations in the southern United States. Seed and seedling characteristics may provide early selection criteria for genetic improvement in these short-rotation systems. Five experiments with twelve open-pollinated families were used to determine genetic and product-moment correlations and to identify the best traits. Rate of germination, as expressed by "peak value", and root growth potential of one-year-old seedlings (before planting in the field) were correlated with dry weights, number and growth of sprouts, and bud characteristics after one growing season in the field.

Keywords: Platanus occidentalis, early selection, root collar diameter, APA, genetic correlation, root growth potential.

INTRODUCTION

Acceptance of short-rotation energy plantations of woody crop species in the United States can be enhanced by increased productivity from these plantations, provided costs are kept low. These short-rotation plantations (three to five years) represent a unique forest cropping system for geneticists, since the harvested product can be more influenced by the quality of the forest reproductive material planted (seeds, seedlings, or vegetation propagules) than is experienced for longer-rotation systems. Manipulations of the environmental, genetic, and physiological factors affecting the quality, and thus future productivity, of these forest reproductive materials may provide lower cost practices for increasing productivity than do many cultural activities that are done after planting.

American sycamore (Platanus occidentalis L.) is a candidate species for short-rotation energy plantations in the southern United States. Early indirect selection at the seed or seedling stage would save the costs of expensive field progeny tests. Results from germination, germinator, nursery, greenhouse, and field tests were used to address three questions basic to short-rotation genetic studies: (1) can the effects of variable seedling density in the nursery bed be separated from genetic effects for family root collar diameter, (2) how important is root collar size as an indirect measure

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of various growth and root characteristics in the field, and (3) are seed and seedling characteristics genetically correlated with root collar diameter and early field performance?

METHODS

Test Designs and Measurements

Data were used from twelve open-pollinated sycamore families that were common to germination, germinator, nursery, greenhouse, and field tests.

The germination test consisted of four replications of 50 seeds each from each open-pollinated family seed lot. The seeds were placed on Kimpack medium in germination incubators, and germination was measured over a 21-day period under a temperature-light regime of eight hours of light at 30°C and sixteen hours of dark at 20°C.

The germinator test was arranged in a randomized complete block design with four replications and 50 seeds per family plot within each replication. Seeds were placed at a 3.7-by-3.7cm spacing on Stultz white germination paper in a germinator. Measurements were taken for germination and hypocotyl lengths on eleven dates over a 97-day period. Light and temperature regimes were the same as for the germination test.

Seeds in the nursery test were planted in a 1.2-m-wide by 30.5-m-long nursery bed. A randomized complete block design with three replications and border rows was used. Each family plot within a replication consisted of 96 planting spots spaced at 10-by-10 cm on eight rows across the bed by 12 columns along the bed. Three to four seeds were planted at each spot and thinned to one after measurement of survival on the 33rd day after planting. Seedling heights and root collar diameters were measured at monthly intervals from June to January (58 to 264 days after planting).

Twenty seedlings from each family in the nursery were lifted on the 264th day, measured for root characteristics, and planted in four-liter containers of sand on ground-heating mats under mist and supplemental lighting in a greenhouse. A completely random experimental design was used. Five of the 20 seedlings per family were chosen to have 8-10 mm root-collar diameters and were designated for "border" containers. The other 15 seedlings per family were selected to have five with "large" root collars (12-mm diameter), five with "medium" root collars (9-mm), and five with "small" root collars (6-mm). These were used to test family and root collar size effects on root growth potential (RGP). RGP was measured as the number of short (1.0-2.5 cm), medium (2.5-5.0 cm), and long (>5.0 cm) new white roots after 21 days in the greenhouse.

The 240 seedlings from the RGP study were transplanted from the greenhouse to a field site in mid February (300-day-old seedlings). The same completely random design was used. Measurements of survival and growth were taken in May. After one year in the field, the seedlings (now two-years-old from seed) were dug up and measured for root and stem dimensions and for dry weights.

Calculations

APA-Adjusted Nursery Root Collar Diameters Family differences in root collar diameter at time of lifting from the nursery bed were adjusted for differences in seedling density by using a geometrically derived covariable, "area potentially available" (APA). The APA index was calculated for each seedling using a Fortran computer program developed by Nance and Grissom (1988). The procedure involves the geometric construction of unweighted polygons that circumscribe each seedling. Sides of the polygons are placed half way between the subject seedling and its nearest competitors. The area of the polygon is calculated to determine the area potentially available for growth of the seedling. A detailed explanation of the APA formulation is given in Nance et al. (1987). Analyses of variance for both APA-adjusted and unadjusted root collar diameters were conducted.

Peak Value Peak value (PV), a measure of rate of germination, was calculated after Czabator (1962):

PV = peak value, the maximum value of the cumulative percent germination for each day of the trial divided by the corresponding number of days from the beginning of the trial.

Correlations Product-moment correlations of family means were calculated between 20 selected field traits and 38 selected germinator, nursery,and greenhouse traits. Since the germinator and nursery test designs differed from the greenhouse and field design, clean genetic correlations could not be determined between early seed and seedling traits and later field traits. However, the product-moment correlations serve as measures of the intensity of associations between the traits and are considered to be composed of genetic, maternal, and common environment effects.

Genetic Correlations Genetic correlations were calculated for those pairs of field and greenhouse traits that showed significant product-moment correlations. Each pair of traits was measured on the same individual seedling that was lifted from the nursery bed, planted in a container in the greenhouse, and later transplanted to the field.

Components necessary to calculate the genetic correlations were acquired from analyses of variance and covariance on the half-sib families. Genetic correlations were calculated as:

$$r_G = \frac{COV_{xy}}{\sqrt{VAR_x * VAR_y}}$$

where VAR and COV refer to the components of variance and covariance for half-sib families and x and y to a field trait and greenhouse trait.

RESULTS AND DISCUSSION

Nursery Bed Root Collar Diameter (RCD)

Families were significantly different for unadjusted RCD and not

different for APA-adjusted RCD (Table 1). Competition among nursery seedlings for available root growth space was considered an important factor in RCD differences. Where localized seedling density varied, performance was biased toward seedlings that had less root competition and more available root growth space. Individuals within a family were planted in block plots within replications. When empty spots occurred within a block many seedlings of the same family benefited. Families with low percent-germination had large diameter seedlings. The effect of increased available area is removed in the APA-adjusted analysis, and families no longer exhibited significant differences in RCD. The significant unadjusted family differences indicated that RCD was under environmental rather than genetic control.

Table 1. Analysis of variance for root collar diameter at time of nursery lifting for both unadjusted and APA-adjusted models.

		Unadjusted		Adjusted	
Source of Variation	DF	MS	F-test	MS	F-test
APA (covariable)	1			0.9424	16.37**
Rep	2	12.8929	71.38**	2.8632	6.44**
Fam	11	1.2478	2.88**	0.4816	1.08
Rep*Fam	22	0.4327	2.40**	0.4429	5.73**
Within plot (Error)	1180	0.1806		0.0776	

*Significant at the 0.05 level.
**Significant at the 0.01 level.

The sequential mean square F-test for the APA covariable implies that APA alone can account for a significant amount of variation in RCD. Variation in seedling density due to mortality in the nursery bed has long been a problem in analyzing nursery experiments. The APA covariable analysis was useful in accounting for variation due to inconsistent spacing, and it should be tried in other nursery studies.

The replication-by-family interaction terms were significant in both unadjusted and adjusted analyses. Family rank changes across replications were observed, but three of the 12 families did show rank stability.

Seed and Seedling Correlations

Peak value (PV) for germination of open-pollinated seed lots is significantly correlated with first-year field growth, crown growth potential (bud characteristics), and biomass (dry weight) (Table 2). PV is a measure of a seed lot's general vigor, and in this case seed vigor has correlated well with early plant performance in the field. Identifying families with good PV may allow early identification of genotypes to place in an accelerated breeding program.

Greenhouse root growth potential (RGP) traits and number of medium lateral roots produced in the nursery correlated well with number and growth of secondary buds, number of total apical buds, and growth of all buds in the field test. These results suggest that the above and below ground numbers of potential lateral meristems (or branching pattern) may be reflections of each

Table 2. Significant product-moment correlations between germination, nursery, greenhouse, and field traits for 12 open-pollinated sycamore families.

Field Trait	Germination Trait	Greenhouse RGP Traits No. of New Roots			Nursery
	Peak Value	Long	Medium	Short	Medium Lateral Roots
Stem Dry Weight	0.596*	-.096	.035	-.008	.032
Root Dry Weight	0.738**	-.026	.125	.099	.279
Total Dry Weight	0.683**	-.071	.075	.037	.137
Growth Secondary Buds (3 months)	0.805**	0.590*	0.818**	0.821**	0.678**
No. Secondary Buds (1 year)	0.586*	0.529	0.711**	0.805**	0.641*
Length of Topmost Sprout (3 months)	-0.714**	-.219	-.454	-.333	-.514
No. Total Apical Buds/ Tree (1 year)	0.560*	.527	.717**	.817**	.614*
Growth All Buds/Tree (1 year)	0.778**	.543	.700**	.702**	.489
Root Volume (1 year)	0.624*	-.120	.002	.019	.112

* Significant at the 0.05 level.
** Significant at the 0.01 level.

other. RGP traits might be useful for early identification of families that will produce large crowns and more photosynthetic area.

Root Collar Class

Analyses of variance on 35 nursery, greenhouse, and field traits resulted in 25 analyses for which root collar class had a significant effect (Table 3). Significant family effects were also found for eleven of these traits, but in no case was there a significant family-by-root-collar-class interaction. These results support the hypotheses that (1) RCD is the single most important morphological measure of early growth potential and (2) genetic differences in growth potential exist for seedlings of the same RCD. Effective early

selection in the nursery will require either (1) equal growing space per seedling or (2) evaluation of only seedlings having the same root collar class.

Table 3. Nursery, greenhouse, and field traits that showed significant root collar class (RCC) or family differences.

Traits	Study Mean	Significance Family	RCC	Family*RCC
Nursery				
Nursery height (cm) day 58	7.7	**	**	NS
Nursery height (cm) day 93	31.5	**	**	NS
Nursery height (cm) day 120	51.8	**	**	NS
Nursery height (cm) day 156	77.7	NS	**	NS
Nursery hegith (cm) day 181	79.7	NS	**	NS
Nursery RCD (mm) when lifted	9.2	NS	**	NS
Root vol. (ml) when lifted	21.7	NS	**	NS
No. medium lat. roots-lifted	3.8	NS	**	NS
No. large lat. roots-lifted	1.1	NS	**	NS
Taproot length (cm)-lifted	28.9	*	**	NS
Greenhouse				
No. long new roots 21-days	4.8	++	NS	NS
No. medium new roots 21-days	12.7	**	NS	NS
No. small new roots 21-days	25.5	NS	NS	NS
Field				
Total no. sprouts/tree	8.5	**	**	NS
Length top sprout (cm)-May[a/]	2.3	NS	**	NS
Growth sprouts/tree (cm)-May	12.6	**	**	NS
Height (cm)-Jan.[b/]	98.1	NS	**	NS
Groundline diam. (mm)-Jan.	16.8	NS	**	NS
Total no. apical buds/tree-Jan.	7.2	**	**	NS
No. 2° apical buds/tree-Jan.	5.9	**	**	NS
Tot. growth (cm) 2° buds	104.5	NS	**	NS
No. large lateral roots-Jan.	1.3	NS	**	NS
No. small lateral roots-Jan.	5.8	*	**	NS
Tot. no. lat. roots @20cm radius	8.7	NS	**	NS
Stem dry wt.(gm)-Jan.	47.4	NS	**	NS
Root dry wt.(gm)-Jan.	40.5	NS	**	NS
Severe dieback (0=no,1=yes)-Jan.	0.2	NS	**	NS
Slight dieback (0=no,1=yes)-Jan.	0.7	NS	**	NS

[a/]Third month in the field (15-months-old).
[b/]Twelfth month in the field (2-years-old).
++ Significant at the 0.07 level.
* Significant at the 0.05 level.
** Significant at the 0.01 level.

Genetic Correlations

The number and growth of sprouts (elongation of buds) and number of total and secondary apical buds in the field all showed strong genetic correlations with root growth potential (Table 4). No correlation was found between primary apical buds and RGP, however. These results suggest that some families are allocating more of their first-year growth to limbs than others. In subsequent years those families with a larger crown size would possess a greater photosynthetic surface area. Like the product-moment correlations, the genetic correlations indicate that families with high RGP's may build larger crowns faster than those with lower RGP's, even though primary bud growth (first-year height growth) may not differ.

Table 4. Genetic correlations for measures of root growth potential (RGP) and taproot length with first-year field performance of 12 open-pollinated sycamore families.

Field Trait by Greenhouse Trait	Genetic Correlation
No. Sprouts/Tree (after 3 months) by	
Taproot length (day 264)	-0.764
No. of long (>5 cm) new roots (day 21)	0.990
No. of medium (2.5-5.0 cm) new roots (day 21)	0.966
No. of short (1.0-2.5 cm) new roots (day 21)	1.138
Growth all sprouts/tree (after 3 months) by	
No. of long (>5 cm) new roots (day 21)	0.953
No. of medium (2.5-5.0 cm) new roots (day 21)	0.970
No. of short (1.0-2.5 cm) new roots (day 21)	1.093
Total No. Apical buds/tree (after 1 year) by	
No. of long (>5 cm) new roots (day 21)	0.852
No. of medium (2.5-5.0 cm) new roots (day 21)	1.002
No. of short (1.0-2.5 cm) new roots (day 21)	1.374
No. secondary apical buds/tree (after 1 year) by	
No. of long (>5 cm) new roots (day 21)	0.834
No. of medium (2.5-5.0 cm) new roots (day 21)	0.976
No. of short (1.0-2.5 cm) new roots (day 21)	1.339

Taproot growth showed a strong negative genetic correlation (-0.764) with the number of sprouts per tree. This could be the result of some families diverting energy from bud development to taproot elongation.

SUMMARY AND CONCLUSIONS

1. Family differences in seedling root collar diameters (RCD) are interpreted to be a result of differences in seedling density in the nursery bed, rather than genetic differences. The "area potentially available" (APA) for each seedling appears to be a useful measure of localized

nursery bed density, and it is a promising tool for future nursery experiments.

2. Root collar diameter was the most important morphological measure of early field performance potential. However, family differences in root morphology, root growth potential, and field performance for seedlings of the same root collar class were also found. Effective early selection in the nursery for genetic improvement will require equal growing space per seedling or use of seedlings of equal root collar class.

3. Large positive genetic correlations suggest that families with high RGP's are more likely to build large crowns faster and produce more photosynthetic area than those with lower RGP's.

4. Czabator's peak value for germination of open-pollinated family seed lots was highly and positively correlated with eight first-year field traits. This seed trait may allow early screening to reduce the number of families that are progeny tested for short-rotation energy programs.

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CONCURRENT SESSION: 2B

GENETIC PERFORMANCE AND EARLY-AGE SELECTION WITHIN A TEN-YEAR OLD MID-WEST SYCAMORE PROGENY TEST

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Abstract.--Geographic seed source adaptability, family performance and early-age selection were investigated in a ten-year-old open-pollinated American sycamore test located in southern Illinois. Low genetic (r_g = 0.06) and phenotypic correlations (r_p = 0.44) between age-1 height and age-10 volume indicate the uselessness of age-1 height as a correlated selection trait. Large genetic (r_g = 0.96) and phenotypic correlations (r_p = 0.73) between age-6 height and age-10 volume suggest the use of age-6 height as early-age selection trait. Estimates of indirect gain for age-10 volume obtained through coefficients of genetic prediction (CGP) and correlated response (CR) were highest from selection of age-7 height (e.g. CGP = .131, CR = 18.0%). In terms of genetic gain per unit of time age-6 height provided the optimal selection differential for age-10 volume.

The Alabama and South Carolina seed sources were taller than the Kentucky source at age 1. This ranking was reversed by age 2 and has held stable through age 10. The Kentucky source produced the highest total volume, yielding approximately 11 percent more volume per acre than the Alabama source and 20 percent more than the South Carolina source.

Keywords: Platanus occidentalis L., early-age selection, coefficient of genetic prediction, correlated response.

INTRODUCTION

Increased genetic gains per unit of time will result from early-age selection of sycamore (Platanus occidentalis L.). Rapid juvenile growth culminating in a 15-year pulpwood rotation is expected when sycamore is grown on rich alluvial soils. Short rotations accentuate the need for effective early-age selection strategies. Various methods have been used to determine effective early-age selection by many authors including Foster (1986a), Loo and others (1984), Lambeth and others (1983), and Squillace and Gansel (1974). Two methods, correlated response (CR) (Falconer 1960) and coefficients of genetic prediction (CGP) (Baradat 1976), were used in quantifying early-age selection efficacy. Increasing the efficacy of early-age selection in sycamore also provides the breeder the option of clonal propagation. Thus, shorter test duration, increased gains per unit of time, and the ability to clonally propagate selections emphasize the necessity for early-age selection in sycamore.

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The purpose of this study was to determine seed source differences, genetic variability among families and provide information on optimal selection age.

METHODS

Open-pollinated seed from 27 sycamore mother trees was collected and grown at the Kentucky State Nursery, Gilbertsville, Kentucky during 1977. Mother tree collections were grouped into three geographic sources: South Carolina Coastal Plain (SC), central Alabama (AL), and west Kentucky, west Tennessee and southern Illinois (KY). Seedlings were outplanted as 1-0 stock on a secondary bottomland tract in Pulaski Co., Illinois. The area was formerly timbered, logged in 1976, site prepared in 1977, and row-marked and slit at a spacing of 11 x 11 feet in December 1977. The Soil Conservation Service described the test site soils as Bonnie and Belknap silt loams. They are deep but poorly drained soils derived from sediments washed off of loess uplands. The test was planted on April 3, 1978. The test design was a randomized complete block consisting of six blocks, three geographic source groups, a total of 27 open-pollinated families, and 10 tree row-plots (Table 1). Families were grouped by seed source to facilitate estimates of volume on a per acre basis.

Total height was measured at ages 1, 6, 7, and 10, while diameter at breast height was taken at ages 7 and 10. Individual total tree volumes (ft^3) were calculated for ages 7 and 10 using the equation volume = .00252 (D^2H), where D = diameter breast height and H = total height, developed by Belanger (1973) for plantation-grown sycamore.

Analysis of variance and multivariate analysis of variance were done on an individual tree basis for all traits. Variance components were estimated by equating mean squares or mean cross products with expected mean squares. Geographic source effects were considered to be fixed while all other effects were considered to be random.

Table 1. Analysis of variance and expected mean squares for the 1978 Sycamore Test.

Source	d.f.	Expected Mean Squares
Block	(b-1)	
Geographic Sources	(s-1)	$\sigma^2_\omega + n\sigma^2_{BF/S} + bn\sigma^2_{F/S} + fn\sigma^2_{BXS} + bfn\theta_S$
Block*Geo. Sources	(b-1)(s-1)	$\sigma^2_\omega + n\sigma^2_{BF/S} + fn\sigma^2_{BXS}$
Family/Geo. Sources	(f-1)s	$\sigma^2_\omega + n\sigma^2_{BF/S} + bn\sigma^2_{F/S}$
Block*Family/Geo.Sources	(b-1)(f-1)s	$\sigma^2_\omega + n\sigma^2_{BF/S}$
Error	bsf(n-1)	σ^2_ω

Estimates of narrow-sense heritabilities and genetic and phenotypic correlations were calculated from the estimated components of variance and covariance (Becker 1975). Coefficients of genetic prediction (CGP) were derived from procedures outlined in Baradat (1976) and van Buijtenen and Tuskan (1986). The indirect gains estimated by the correlated response (CR) were derived from Falconer (1960).

RESULTS AND DISCUSSION

Test survival has remained fairly stable from age 2 (91%) through age 10 (90%). Total height at ages 1, 2, 6, 7 and 10 for the entire test averaged 4.7, 24, 29, and 46 ft., respectively. Average dbh and volume were 3.8 in. and 1.17 ft^3, respectively at age 7 and 5.4 in. and 3.61 ft^3, respectively at age 10. The overall slower growth of this test, relative to growth expected on alluvial soils, is indicative of less favorable soil moisture and lower fertility of secondary bottoms.

No significant survival differences were detected among the three sources through time. At age 10 all three sources averaged approximately 90 percent survival. Age-1 height of the SC source (4.8 ft.) was slightly taller than the AL (4.7 ft.) and KY (4.6 ft.) sources, however by age 10 this pattern was reversed. Average height, dbh, and volume at age 10 were greater for the KY source (47 ft., 5.6 in. and 4.0 ft^3) followed by the AL (46 ft., 5.4 in. and 3.6 ft^3) and SC (45 ft., 5.1 in. and 3.2 ft^3) sources, respectively.

Knowledge of performance of various geographic seed sources within the mid-west is imperative in defining seed source limitations. Seed source differences were nonsignificant for height at all ages. However, seed source differences were shown for dbh and volume at ages 7 and 10. Non-significant seed source differences for height at all ages contrasts with what Schmitt and Webb reported (1971), showing significant seed source effects for age-3 height in a sycamore progeny test in Mississippi. However, Land (1981), Nebgen and Lowe (1985), and Wells and Toliver (1987) found no evidence of seed source effect for height growth of sycamore. Near optimal alluvial site conditions, including the moderating effect of a large riverine system, such as the Mississippi River, seems to allow greater movement of seed sources. In contrast, secondary bottomland sites, such as this test site, are less favorable and result in poor growth of sources adapted to milder climates. The excellent performance of the near local KY source indicates that for the southern Illinois area seed movement should be restricted to within 100 miles south of the planting site.

Highly significant differences among families-within-seed sources were shown for height at all ages (Table 2). This component of variation increased as age increased accounting for 5.4 percent of the total variation at age 1 and 7.0 percent at age 10. The block by family-within-seed source components were fairly large and highly significant for all traits at all ages. Tree-to-tree differences accounted for most of the variation associated with all traits through age 10. Tree-to-tree differences were lower for height than either dbh or volume.

Table 2. Variance components, percent of total variation (in parentheses) and significance levels for all traits .

Traits	Family/Source	Block X Family/Source	Within Plot
Age 1 Height	0.0184[ns a/] (1.6)	0.1036** (9.1)	1.0207 (89.3)
Age 6 Height	0.5433**[b/] (5.4)	1.3957** (13.9)	8.0819 (80.7)
Age 7 Height	0.6191** (5.9)	1.3771** (13.0)	8.5769 (81.1)
Age 10 Height	1.2811** (7.0)	2.0043** (10.9)	15.0328 (82.1)
Age 7 DBH	0.0220* (2.8)	0.0579** (7.4)	0.7021 (89.8)
Age 10 DBH	0.0191[ns] (1.5)	0.0330* (2.6)	1.2117 (95.9)
Age 7 Volume	0.0129* (2.9)	0.0390** (8.8)	0.3913 (88.3)
Age 10 Volume	0.0556[ns] (2.0)	0.1160** (4.1)	2.6429 (93.9)

[a/] ns = non-significance at the .05 level of probability.
[b/] * = Significant at the .05 level of probability; ** = significant at the .01 level of probability.

Family and individual tree heritabilities for height increased from age 1 to age 10 (Figure 1). Family heritabilities were consistently larger than individual tree heritabilities. Both family and individual tree heritabilities are somewhat inflated due to the single test site. However, the relative performance continues to widen between the good and poor families and individuals indicating their larger role in the observed total variation. Family and individual heritability estimates for dbh and volume decreased between ages 7 and 10 (Figure 1). Increasing tree-to-tree variation, attributed to microsite variation and the highly variable genotypic response to the early disease incidence, has resulted in decreased heritability estimates.

Genetic and phenotypic correlations decreased as the age difference increased (Figure 1). Genetic correlations for height, dbh and volume were usually larger than phenotypic correlations. Age-1 height was poorly correlated with height, dbh and volume at ages 7 and 10 (Figure 1). However genetic and phenotypic correlations between age-6 height and age-10 height were extremely high. Because these correlations are a comparison of performance within a specific growth phase in particular, the juvenile phase they were expected to be high (Namkoong and Conkle 1976). The low correlations involving age-1 height and later age traits are probably the result of nursery effects and the early disease situation. These effects demonstrate the inefficacy of using age-1 height as a selection trait for gains at age 10. However, correlations between age-6 and age-10 traits were excellent indicating the possible use of it as a selection age.

The CGP calculated between age-1 height and later measurements of height, dbh and volume tend to be low and decrease with increasing age (Figure 1). The CGP between age-1 height and age-10 volume resulted in a value (.004) much lower than the .08 value shown for individual heritability of age-10 volume. The lower CGP indicates that response in age-10 volume from selection of age-10 height would be very low compared to direct selection on age-10 volume. In contrast, the CGP's calculated between traits at ages 6, 7 and 10 were either very

	H1	H6	H7	H10	D7	D10	V7	V10
H1	0.36 (0.06)	0.46	0.31	0.40	0.18	0.23	0.01	0.06
H6	0.53	0.63 (0.22)	0.97	0.89	0.91	0.97	0.93	0.96
H7	0.49	0.93	0.65 (0.25)	0.94	0.86	0.92	0.89	0.96
H10	0.37	0.78	0.83	0.71 (0.28)	0.77	0.90	0.82	0.93
D7	0.52	0.82	0.82	0.72	0.51 (0.11)	1.02	0.97	0.93
D10	0.43	0.73	0.74	0.73	0.90	0.40 (0.06)	1.01	0.93
V7	0.52	0.80	0.80	0.68	0.96	0.83	0.51 (0.12)	1.00
V10	0.44	0.73	0.75	0.76	0.90	0.97	0.88	0.48 (0.08)

Figure 1. Additive genetic correlations (above the diagonal), phenotypic correlations (below the diagonal) and family and individual (in parentheses) heritabilities (along the diagonal).

	H1	H6	H7	H10	D7	D10	V7	V10
H1	(.06)	.054	.039	.053	.015	.015	.001	.004
H6		(.22)	.218	.219	.142	.111	.147	.128
H7			(.23)	.241	.137	.109	.147	.131
H10				(.28)	.137	.117	.148	.148
D7					(.11)	.085	.111	.059
D10						(.06)	.085	.064
V7							(.12)	.098
V10								(.08)

Figure 2. Coefficients of genetic prediction (CGP) and individual tree heritability estimates (in parentheses) for all traits and ages in the 1978 Sycamore Mother Tree Test.

similar or exceeded the estimated heritabilities of the response trait. However, selection based on age-7 height resulted in the highest CGP for volume at age 10. This indirect gain was higher than the CGP calculated for volume at ages 7 and 10. In addition, the CGP computed between age-10 height and volume was higher than age-10 volume heritability. Interestingly, age-10 volume gains resulting from selection of age-10 or age-10 height were larger than those using age-10 dbh or volume. Even the use of age-6 height as the selection trait resulted in greater gains in age-10 volume than selection based on either age-10 dbh and volume. This relationship with height occurs because of the high genetic correlations shown to exist between height at ages 6 and 7 with volume at age 10. In addition, the lower phenotypic covariance for height and volume compared to the phenotypic variance of volume alone also affects this relationship. Thus, the lower environmental effects on phenotypic height relative to phenotypic dbh resulted with a higher CGP with respect to volume.

Indirect gains for age-10 volume were obtained through the use of the correlated response (CR). Selection for height at age 7 resulted in the highest genetic gain (18%) for age-10 volume (Table 3). Genetic gains per unit of time for age-10 volume showed age-1 height the lowest at 0.15 percent and age-6 height the highest at 1.96 percent (Table 3). The extremely high genetic correlations for height, dbh, and volume among ages 6, 7, and 10 have led to a high correlated response for age-10 volume.

Maximizing genetic gains per unit of time through effective early-age selection has been shown by Foster (1986b) and Rousseau (1987) in eastern cottonwood, and Lambeth and others (1983) in loblolly pine. The higher gains resulting from the selection of height differed from Foster (1986b) and Rousseau (1987) where selections of dbh and volume at earlier ages resulted in much higher gains in volume at later ages. The similarities between CR and CGP calculations affirm that selection of either age-6 or age-7 height would result in approximately identical gains for age-10 volume. Both CR and CGP showed that very little gain would result from selection of age-1 height for age-10 volume. The CR of age-6 height was nearly as high as age-7 due to the high genetic correlations shown for age-6 height and age-10 volume. Although similarities were shown between gains at age-10 volume predicted by CR and CGP based on selection of height at ages 6 and 7, genetic gains per unit of time indicate that selection of age-6 height is more efficient. This selection age is very similar to those identified by Nebgen and Lowe (1985). Because of various factors such as the lack of frequent earlier measurements, few measurement traits, and the present young age of studies we are unable to precisely define the optimal selection age and trait for volume in a pulpwood rotation of sycamore. However, it is evident at this time that age-1 height is not an effective selection trait and that selection at or near age 5 will result in reduction of the generation interval. An additional aspect of early-age selection allows the breeder to approach sycamore improvement from the clonal aspect as well as from the traditional seed orchard method.

Table 3. Correlated response and genetic gains per unit of time for age 10 from selection of other traits.

Selected Trait[a]	Correlated Response (ft^3)	Correlated Response (%)[c]	Genetic Gain Per Unit of Time[b] (%)[d]
Age 1 Height	0.0207	0.6	0.15
Age 6 Height	0.6355	17.6	1.96
Age 7 Height	0.6498	18.0	1.80
Age 7 DBH	0.4354	12.6	1.26
Age 7 Volume	0.4889	13.5	1.35

[a] Selection intensity was 5/1360 individuals (i=2.9750).
[b] Unit of time is defined as selection age plus three years.
[c] Percent response - Correlated Response (ft^3)/Average age-10 volume (3.61 ft^3)
[d] Percent Gain per Unit of Time - Percent Response/Selection age plus three years

CONCLUSIONS

This study demonstrates the feasibility of early-age selection in sycamore. A shortened generation interval allows for increased gains per unit of time and the possibility of clonal propagation. Large tree-to-tree variation partially resulting from disease susceptibility led to high phenotypic variation which affected the various estimates of genetic parameters. Significant family-within-seed source differences were shown for all traits. However, significant seed source differences were not shown for height at any age. Family and individual tree heritabilities increased with age for height but decreased for dbh and volume.

Genetic and phenotypic correlations were extremely poor for age-1 height with all other traits. Height at ages 6 and 7 were highly correlated with age-10 volume. Coefficients of genetic prediction for age-10 volume were highest for age-7 height. However, selection of age-6 height for gains in age-10 volume were very similar to selection of age-7 height. In terms of gain per unit of time, the optimal selection trait was age-6 height for the response trait of age-10 volume.

The local seed source (KY) proved to be better adapted to the conditions found on the 1978 test site. This is in contrast to reports of southerly seed sources outperforming local material in tests located on alluvial floodplain sites.

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OPTIMAL AGE FOR SELECTING LOBLOLLY PINE SEED SOURCES

F. H. Kung[1]

Abstract:--Five methods to estimate the optimal age for selection were compared using measurement of total tree height of 15 provenances at 5, 10, 15, 20, and 25 years of age obtained from a south-wide loblolly pine seed source study. The best time to separate seed sources, based on the comparison of F-values, is at age 15, when the heritability is also at its peak. However, because phenotypic variance increases with age, maximum genetic gain at a given selection intensity occurs five years later, at age 20. Based on the annual genetic gain, selection should be at age 10. Correlation between initial growth and periodic growth becomes negative after age 15. Thus, the optimal age for selecting trees for height growth among loblolly pine of different provenances should be between age 10 and 20 years.

Keywords: Pinus taeda L., provenance test, genetic gain.

INTRODUCTION

Loblolly pine is a commercially important species of the eastern United States. Long-term seed source trials have been established in its native range (Wells 1983), at the fringe of its range (Rink and Thor, 1971, Talbert and Strub, 1987), and outside of its native range (Rink and Wells, 1988). In recent years, sudden mortality of individual dominant and codominant trees were observed at about age 23 in southern Arkansas (Wells and Lambeth, 1983), and about age 35 in southern Illinois (Wells and Rink, 1984). The problem of late mortality may have been the manifestation of maladaptation on off-sites. The result of stress necessitates long-term observation of provenance test through a complete rotation.

However, when loblolly pine was planted within its native range, self-thinning occurred between ages 25 and 30 (Schmidtling 1988). Thus, if we did not consider selection for mortality rate, which may be either due to maladaptation or due to self-thinning, but to concentrate only on the single trait of height growth, then, when would be the optimal age for making a seed source recommendation?

The best time to determine selection age is at an age when the differences among seed source are most prominent. Variation among seed sources should be significantly greater than differences among trees within seed sources. In statistical terms, the age with the largest F-value should be the selection age.

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The second method is based on the argument that genetic control should be fully demonstrated at the selection age. Thus, the age with the highest heritability should be chosen. It will be illustrated later in this paper that this method gives the same result as the first method.

For a trait whose variance is not static and changes with time, one may argue that emphasis should be placed on the maximization of selection differential. For a given culling intensity, selection differential should be at its maximum when one make a seed source recommendation. This approach may be impractical for total growth whose variance always increases with time so that one must keep on waiting forever. Furthermore, Because heritability may vary within the life time of the seed source study, one should consider both selection differential and heritability at the same time. Thus, the selection age should be located at the peak of genetic gain, which is the product of selection differential and heritability. This is the third method.

The fourth method follows the above argument but goes a step farther. If one considers the time element, (after all, time is money) then one should place the selection age at the peak of average annual genetic gain. The average annual genetic gain is the genetic gain divided by the age of selection.

The fifth method involves comparing correlation coefficients between periodic increment and the total growth at the beginning of the period and at some putative selection age. If there is a positive correlation between the two growth traits, or the fast growing seed sources are still growing faster than the poor seed sources, it may be worthwhile to wait for the farther separation of seed source performance. On the other hand, if the correlation should become zero, then, waiting would not improve separation of seedlots. Finally, if the correlation should turn from positive to negative, then waiting is wasting of time. Because the longer you wait, the less is the differentiation among seed sources, even to the degree that it may work against the result of early selection.

In this paper, total height of trees in the south-wide loblolly pine seed source study is used to illustrate the five different methods in choosing the optimal selection age.

MATERIALS AND METHODS

The south-wide loblolly pine seed source study represented 15 seed sources in 15 plantations (Wells and Wakeley, 1966). Data used for this paper were extracted from a computer tape which was kindly provided by Dr. O. O. Wells and the Southern Forest Experiment station. I used all available trees measured at age 5, 10, 15, 20, and 25 to calculate the average height for each seedlot in each plantation. These provenance-plantation means then were used as observations in a two-way analysis of variance. The F-value for provenance effects was extracted and from it the heritability for provenance differences was calculated. The standard deviation of phenotypic provenance means were extracted from previous publication (Kung, 1987). The 5-year periodic growth

was computed by the height difference observed in a 5 year interval. Data were analyzed using Statistical Analysis System package programs (SAS, 1985).

RESULTS AND DISCUSSION

Maximum F-value

Using the general linear model for a two-way analysis of variance, the random seed sources effects are significant for all 5 ages. The maximum F-value was found at age 15 (Table 1).

Table 1. F-value, heritability (h^2), standard deviation of provenance means (Sp), genetic gain, annual gain, and correlation (r) between periodic increment and total growth in loblolly pine from age 5 to 25 years.

	Age				
	5	10	15	20	25
F-value	2.33	5.45	6.85*	6.40	4.49
h^2	0.570	0.817	0.854*	0.844	0.777
Sp	0.823	1.592	1.974	2.205	2.227
Genetic gain	0.469	1.300	1.686	1.861*	1.730
Annual gain	0.094	0.130*	0.112	0.093	0.069
r	0.729	0.808	-0.068	-0.129	

* Maximum of 5 values across a row to indicate the optimal selection age.

Using age as independent variable and the F-value as dependent variable in a second order regression, we have,

$$\text{F-value} = -2.432 + 1.126 * \text{Age} - 0.034 * \text{Age} * \text{Age} \qquad R^2 = .9992$$

and the peak F-value of 6.89 is expected at age 16.5 years (Figure 1).

Maximum Heritability

The heritability of provenance means is calculated from the F-value as follows (Kung, 1979):

$$h^2 = 1 - (1 / F)$$

The maximum heritability was found at age 15 (Table 1). The results are the same as method one because heritability is a direct linear transformation of the F-value. However, the second order regression is slightly different from the previous one:

$$h^2 = 0.303 + .0667 * \text{Age} - .00193 * \text{Age} * \text{Age} \qquad R^2 = .949$$

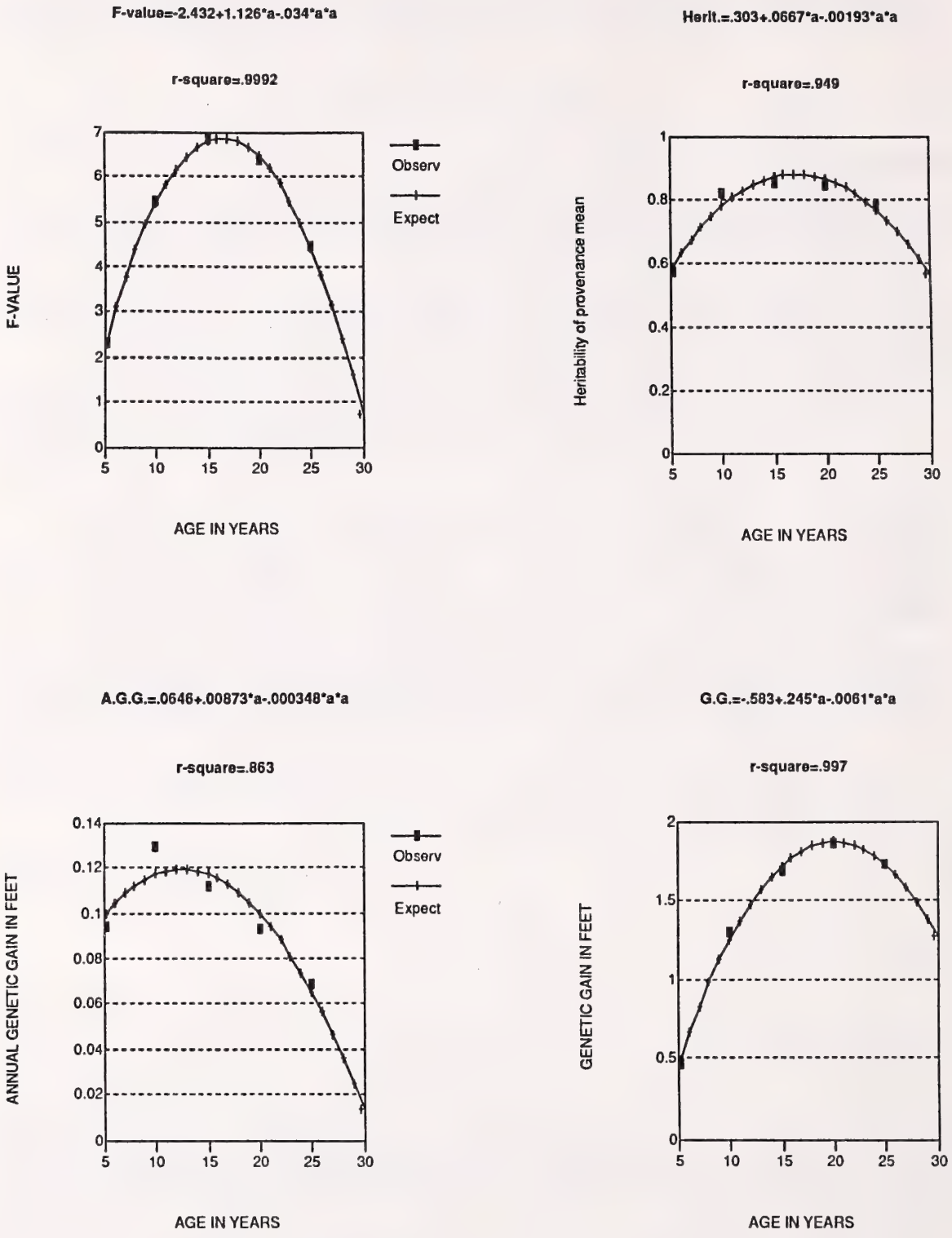

Figure 1. Relationship between age and F-value (upper left), heritability (upper right), genetic gain (lower right), and annual genetic gain (lower left).

The maximum heritability calculated from this function is 0.879 at the age of 17.3 years (Figure 1).

Maximum Genetic Gain

Assuming that the selection intensity (i) is the same for all ages, genetic gain is simply a function of $Sp * h^2$, in feet. The values of genetic gain in Table 1 represent one unit of selection intensity (i=1.0), or 38% of selection proportion. Changing the common selection proportion or selection intensity will change the absolute value for genetic gain but will not change the location for the peak performance. Genetic gain was maximized at age 20 in Table 1. This is the same age calculated from a regression developed from the five pairs of observations:

$$\text{Genetic Gain} = -.5832 + .2447 * \text{Age} - .0061 * \text{Age} * \text{Age} \qquad R^2 = .997$$

Maximum Annual Genetic Gain

Comparison of the five values for annual genetic gain indicates that the optimal selection age is at 10 years. It becomes age 12.5 (Figure 1) if the following regression is considered:

$$\text{Annual Genetic Gain} = .06458 + .00873*\text{Age} - .000348*\text{Age}*\text{Age} \qquad R^2 = .863$$

Correlation Between Periodic and Total Growth

The correlations between the 5-year periodic growth and the cumulative growth at the beginning of a growth period were positive for age 5 and 10, but were negative for age 15 and 20. Thus, the fast growing seed sources are growing faster before age 15, but are slower after age 15. Therefore, optimal selection age should be set at 15, unless growth rates change again after the end point of the present study.

Comparison of Results

The earliest selection age is given by the annual genetic gain criterion (Method 4). Because the annual genetic gain is computed as genetic gain divided by age, choosing this criterion will favor younger ages. In black walnut progeny tests, clonal selection could be made as early as age 4-5 years when the selection efficiency was judged by a ratio of genetic gain per year between early and later years (McKeand, Beineke, and Todhunter, 1979).

The latest selection age is given by the genetic gain criterion (Method 3). Genetic gain is the product of three components: selection intensity, standard deviation of the selection units, and heritability. If we compare this method with methods 1 and 2, given that all three methods have comparable selection intensity and heritability, but with the additional factor in method 3 (phenotypic variance of total growth) which tends to increase with age, then we can see that this genetic gain criterion will favor selection age in later years.

Using the criteria of F-value, heritability, or correlation between periodic and total growth, will favor the selection ages within the mature genotypic phase of stand development. The duration of this phase ranges from 5 to 20 years in loblolly pine and slash pine, 15 to 40 years for Douglas-fir and ponderosa pine (Franklin, 1979).

The transition into negative correlation between periodic and total growth may be explained as follows. As the trees mature sexually, height growth may be subject to restrictions imposed by the need to maintain a competitive position in the canopy and the need to allocate energy to reproductive needs. Hence, height growth may be subject to a population form of developmental canalization (Namkoong, Usnais, and Silen, 1972).

CONCLUSIONS

As illustrated in this paper, the optimal selection age changed slightly with the use of different criteria. For the height growth in loblolly pine, it may be somewhere between 10 to 20 years.

The choice of criterion may be more or less subjective, but judging by costs and benefits, the annual genetic gain criterion seems to be economically sound. If the phenotypic variance were stable over the years (for example, observations were transformed to stabilize the variance), then the three criteria (F-test, heritability, and genetic gain) would have the same results, and the F-value is the simplest to use. The F-value is already available from the analysis of variance table for the provenance test.

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WIDE CROSSING IN LOBLOLLY PINE: CROSSING LIVINGSTON PARISH WITH ATLANTIC COAST AND CENTRAL FLORIDA POPULATIONS

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ABSTRACT

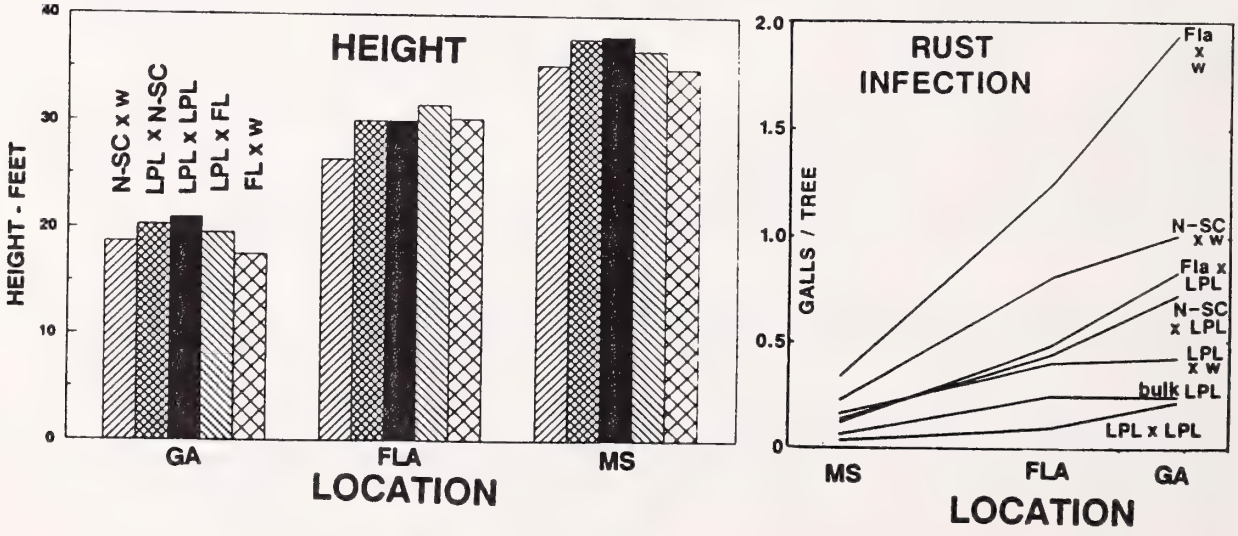
Pollen from five North and South Carolina coastal plain orchard trees, and pollen from five central Florida orchard trees was applied to five Livingston Parish, Louisiana loblolly (LPL) in the Crown Zellerbach orchard near Bogalusa, Louisiana. Also included in the test were open pollinated half-sib progenies from each of the above orchard clones, "wild" LPL trees, and 10 crosses among the LPL orchard clones.

Plantings were established in south Mississippi, central Georgia, and central Florida in January 1977. Seven or eight blocks of each of the 76 treatments were planted in 4-trees plots. Measurements of height at 10 years were analyzed and Fusiform rust at 5 years.

The LPL trees were the most resistant to fusiform rust the Florida trees the most susceptible. The Carolina costal plain trees were slightly less susceptible than the Florida trees. Crosses among the seed sources were intermediate in susceptibility to the parent sources used in the cross. These trends were consistent over all 3 plantings (right figure).

Although the seed source by location interaction was not important, the individual cross (within seed source) by planting interaction was. There was considerable change in rank of individual crosses over the three plantings.

In contrast to the rust infection, seed source and seed source crosses did not perform consistently over all 3 locations in 10th year height (left figure). The main component of this interaction was due to the Florida and Florida X LPL crosses. These did poorly relative to the other seed sources and crosses in south Mississippi and central Georgia, but were the tallest in the central Florida planting. Individual cross (within seed source) by planting interaction was also significant for 10th year height growth.



FIVE YEAR GROWTH RESULTS OF WATER OAK (QUERCUS NIGRA L.) PROVENANCE

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Abstract.--A twelve origin, 68 family seed source study for the western range of water oak (Quercus nigra L.) was established in the field in 1982. Five year growth results are highly significant individual family variation for diameter growth and significant origin variation for height growth. No distinct geographic pattern was evident although the middle Mississippi River and middle eastern Mississippi sources were consistently the better sources for both traits. The southwest Louisiana source was overall the best preforming of the origins for height and growth.

Keywords: Quercus nigra L., provenance, height growth, diameter growth, genetic variation.

INTRODUCTION

Approximately 44 percent of the hardwood volume in the South is composed of various oak species (USDA 1988). Water oak (Quercus nigra L.) is an important component of this oak group. Although water oak is not considered one of the select red oaks as is cherrybark (Q. falcata var pagodaefolia Ell.) and Shumard (Q. shumardii Buck. L.) (Kingsey and Powell 1979), the species is important because of its extensive range, frequency of occurrence and many uses of the wood.

Water oak makes best growth on moist bottomlands and lower slopes of the uplands and is considered to be an intolerant subclimax associate of the bottomland forest (Fowells 1965). The species is not as site sensitive as some of the oaks and can be found making good growth on drier sites as a component of loblolly and shortleaf pine stands. Water oak does not tolerate inundation levels that its close relative willow oak (Q. phellos L.) does.

There is little published genetic information available on water oak. However, there are several progeny test in progress. Both the North Carolina State University-Industry Cooperative Research Program (Purnell and Kellison 1983) and the Western Gulf Tree Improvement Cooperative (Byram and et al. 1987) have some material in progeny test. These programs combine water and willow oak into a single group (water/willow).

Because there was almost no information available on water oak, this species was selected for study. The initial effort was provenance study of the western portion of the range of the species to look at genetic variation within the region for the species. Genetic variability among and within

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stands was also to be addressed, as was establishment techniques, cultural requirement, and maintenance problems associated with genetic test of the species.

METHODS AND PROCEDURES

In the fall of 1978, water oak acorns were collected from the western portion of the natural range of water oak. Twelve origins were located on approximately a 160 X 160km grid (Figure 1). Within these areas two distinct stands were selected and the first three trees having an acorn crop were collected. Sixty-eight family collections were made.

Acorns were cold, moist stored until the spring and were then planted in Spencer-Lemaire rootrainers for initial germination. After sufficient seedlings had germinated they were transplanted to outside nursery beds and planted on a 12 X 12cm (70 seedlings/m^2) spacing.

The seedlings were grown in the nursery for two years. Bare root 2-0 stock was planted at two locations in February 1982. Height and groundline diameter measurements were taken at the end of the nursery phase. At age five in the field, height and diameters (DBH) were collected. One plantation was lost to natural causes during this period and this paper reports on the results of one outplant location at Vernon, Louisiana.

The design of the provenance study was a randomized block with a hierarchal arrangement of 12 locations, two stands within location and three trees within stand. The Statistical Analysis System (SAS 1982) was used to analyze all data.

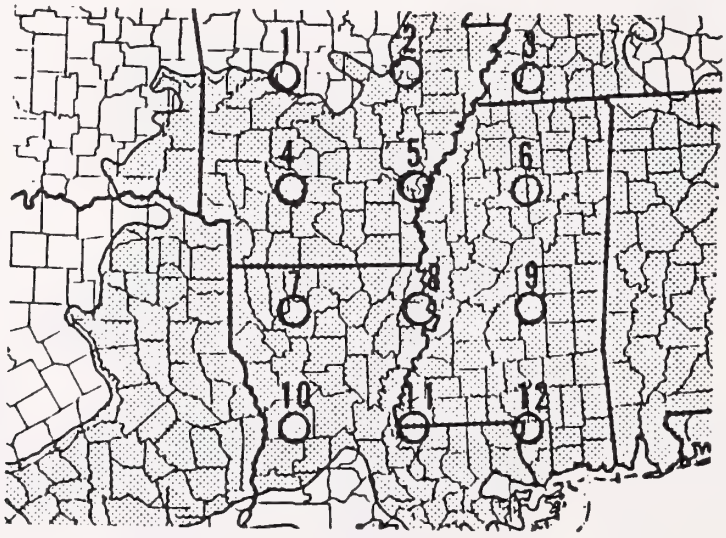

Figure 1. Western range of water oak and seed source collection areas (circles indicate areas) for the water oak provenance study.

RESULTS AND DISCUSSION

Survival was good (80 percent) in one plantation and extremely poor (< 59 percent) in the other. Therefore for the fifth-year results only the Vernon, Louisiana planting was used. During the first-year in the field problems were encountered with transplant shock (Adams 1988). However, seedlings recovered and assumed the approximate height and diameter rankings that existed at the end of the nursery phase.

The initial poor performance, the result of transplant shock, was followed in the subsequent years by good growth. Average height after the fifth year for the plantation was 3.75m and average diameter (DBH) was 3.58cm.

Genetic Variation

Among possible sources of variation, only individual family variation for diameter (DBH) ($P<0.01$) and origin variation for height ($P<0.05$) were significant (Table 1). There were no significant differences for origin or stand/origin variation for diameter. There were no significant differences found for stand/origin nor individual family variation for height growth. Also interaction were not significant.

Variational patterns were difficult to isolate. However, four sources (5, 6, 7, 8) located in the middle and east (Greenville, MS and 100 miles east; Vicksburg and 100 miles east) have consistently been among the best in height and diameter (Figure 1). The best origin for height growth, however, is the southwest Louisiana source (Fort Polk area). There is apparently no geographic pattern for diameter but the southwest Louisiana is also ranked number two for this trait.

Since origin variation was most pronounced for height and no within origin variation was noted, the families within origin appear to be more closely related and thus within origin selection gains for height will be more difficult. Selections, however, from the best seed sources should provide genetic improvement for height. The converse is true for diameter which is highly variable among individual families but there are no detectable origin difference. Selection gains should relatively easy because of the widespread variability of this trait.

Another characteristic not measured but observed, was the difference in leaf initiation in the spring between northern and southern sources. The northern Arkansas sources were 7-10 days later than the southern sources. Once during the past five years a very late freeze has severely damaged foliage of local and the southern sources. The later northern sources were not affected as leafing had not started.

CONCLUSIONS

Based on five years of field data there appears to be considerable individual variation for diameter growth. There does not appear to be a large amount of variation for individual tree height. However, there is a signifi-

cant origin difference for height which would indicate genetic gains could be made by concentrating selection in the best seed source areas.

Table 1. Nursery height, fifth year height and fifth year diameter (DBH) for a 12 origin, 69 family water oak provenance study.

Origin	Height 2-0 Nursery	Height 5th Year Field	Diameter 5th Year Field
	(m)	(m)	(cm)
1	1.05de[1]	3.52bcd	3.99ab
2	1.18abc	3.69abcd	3.72ab
3	0.99e	3.20d	4.35a
4	1.01de	3.41cd	3.29ab
5	1.02de	3.71abcd	3.36ab
6	1.02de	4.01abc	3.45ab
7	1.10bcde	3.66abcd	3.13b
8	1.08cde	3.74abcd	3.57ab
9	1.09cde	4.11ab	3.68ab
10	1.23a	4.23a	4.05ab
11	1.12abcd	3.46bcd	3.19b
12	1.21ab	3.76abcd	3.36ab

[1]Means followed by the same letter in a column are not significantly different at the 0.05 level.

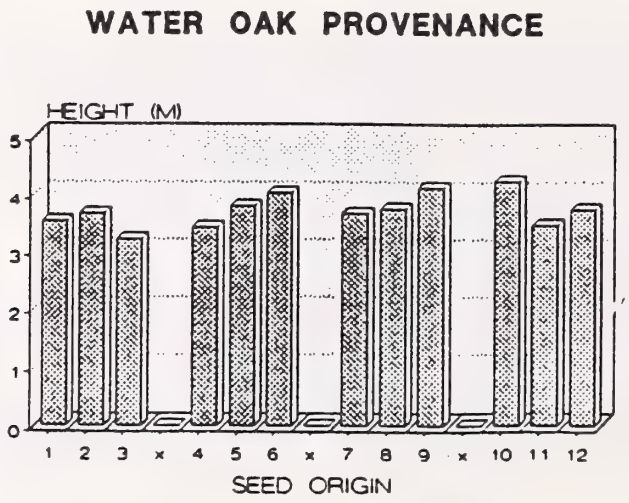

Figure 2. Mean height of 12 water oak seed origins. Low origin number = northern sources, high origin number = southern sources.

Several observations have been made while working with this species. They are:

1. The species exhibits dieback which can cause establishment problems (Adams 1986),
2. The species is fast growing once established,
3. The species is distinct but is confused with several other oak species and at least one hybrid,
4. The species will grow well on a variety of sites, and
5. Growth improvements can be made by selection from the best origins for height and best individuals for diameter.

ACKNOWLEDGMENTS

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APPLICATIONS OF BEST LINEAR PREDICTION TO THE ANALYSIS OF FIVE FULL-SIB LOBLOLLY PINE PROGENY TESTS

by

T. La Farge

Abstract.--Two applications of Best Linear Prediction (BLP) were demonstrated and compared. One method obtains variance and covariance components (homogeneous second moments) from a combined ANOVA based on theory and expected values to predict breeding values for all parents in all environments in a breeding zone. The other method combines variances of family means from separate ANOVAS from each test with family mean correlations between tests to obtain heterogeneous second moments. When there are large environmental differences and genotype x environment interactions such that there are distinct breeding zones, the second method may be used to predict the breeding values of those parents best suited for specific target environments.

Keywords: Pinus taeda L., Best Linear Prediction, full-sib progeny test, type B family mean correlation, breeding value.

INTRODUCTION

Anyone who has worked in a large progeny testing program recognizes the need for appropriate, powerful and robust systems for analyzing progeny test data and estimating breeding values for roguing seed orchards and making selections for second- and future-generation seed orchards. Although well established statistical methods exist for analyzing such data for a wide range of mating and experimental designs, these traditional methods assume balanced data sets at all levels of replication. When these equalities are seriously violated, traditional methods do not work well without costly and time-consuming adjustments, such as missing plot estimates.

Animal breeders have long been acutely aware of this problem, since the data sets they work with rarely have equality of replication. Balanced data sets are almost unachievable, since different herds are not equal in size, and it is not practical to equalize them. Therefore, animal breeders have been compelled to seek methods in which selection could be accomplished in very unbalanced data (Henderson 1984, White and Hodge 1989). The two most

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recently developed methods are Best Linear Prediction (BLP) and Best Linear Unbiased Prediction (BLUP). The utility of both methods is currently growing due to the increasing availability and power of computers, especially of personal computers. This paper will be concerned only with BLP and its analysis on a Compaq 386 personal computer, since BLUP requires a more powerful computer system for any large data set.

METHODS AND MATERIALS

The Essential Components of the BLP Equation

The methods used in these analyses are described by White et al.(1986) for half-sib tests and by White and Hodge (1989) for many other applications, including full-sib progeny tests. The latter source should be consulted for any questions concerning the theory of BLP. A principal utility of BLP is that it can be used to evaluate the performance of parental genotypes in a full-sib progeny test.

A principal assumption of BLP is that the first and second moments are known (Henderson 1984, White et al. 1986). Second moments are specified in two matrices, C and V. The C matrix is a nonsymmetric matrix which defines the genetic relationships between the observed full-sib family means at each site and the true but unknown breeding values, g. Each column of C represents a parental breeding value to be predicted. The elements comprising C are calculated from genetic theory (White et al. 1986).

The V matrix is a symmetric matrix which represents the variances and covariances between the observed phenotypic values. The main diagonal comprises variances of family means for each planting location. In full-sib progeny tests the covariances in the off-diagonals that are not zero are covariances between family means which refer to either: (1) different tests with two common parents; (2) different tests with one common parent; or (3) the same test with one common parent (White and Hodge 1989). Once these matrices have been specified, breeding values can be predicted by means of the following formula:

$$\hat{g} = C'V^{-1}y \qquad (1)$$

where C and V are defined above, y = a data vector of observed deviations of the family means at each location from the location mean, and $\hat{g}$ = the breeding values to be predicted.

The Data Analyzed

The trait considered in the following analyses was total height at age 5, and observations were full-sib family means at each site. All data were collected from four loblolly pine (Pinus taeda L.) progeny tests in southern and one in northern Mississippi. Test locations were as follows:

Test Number	Ranger District	National Forest	Number of Full-sib Families	Number of Check Lots
070001	Black Creek	Desoto	20	4
070002	Strong River	Bienville	26	4
070004	Homochitto	Homochitto	28	4
070005	Strong River	Bienville	28	4
070006	Holly Springs	Holly Springs	29	5

Tests 070001 and 070002 were planted in 1978, and tests 070004, 070005 and 070006 were planted in 1979.

The numbers of families plus check lots that any two tests had in common ranged from a low of 14 to as many as 32. All families comprised seven unrelated 6 x 6 element diallel crossing groups, none of which were complete. The number of crosses per crossing group ranged from two to 13 of the possible 15. Also, tests 070001 and 070002 had four replications, whereas tests 070004, 070005 and 070006 each had three replicates. Hence, considerable imbalance existed in the overall data set.

Two Approaches to BLP

Basically, there are two ways of approaching BLP. One method obtains variance and covariance components (homogeneous second moments) from a combined analysis of variance based on theory and expected values. The other method combines variances of family means from separate ANOVAS for each test with family mean correlations between tests (Type B correlations, Burdon 1977) to obtain heterogeneous second moments. The first method assumes equal variances at all sites and equal levels of genotype x environment interactions between all pairs of sites; the second method is useful if the different sites have unequal variances and assumes different levels of interactions between different pairs of sites. We may obtain breeding values for a target environment which differs in some respect, say elevation, from the other environments being sampled. Both methods are evaluated and compared in the following discussion.

Homogeneous Second Moments

To implement the first method, the five tests were analyzed as a combined ANOVA for total height by means of the VARCOMP Procedure of the Statistical Analysis System (SAS 1987) for Personal Computers. This analysis obtained the variance components needed to derive appropriate variances and covariances to be entered into the C and V matrices included in the BLP equation.

A full-sib family mean of the cross of parents j and k at a single site i is designated Y_{ijk}. The variance of family means from the combined ANOVA of the test was $Var(y_{ijk}) = 1.0088$ with $b = 3.3333$ replicates per test and $n = 8.9738$ trees per plot, harmonic mean basis. The variance of family means comprises all elements on the main diagonal of the V matrix. The covariance of family means with two common parents in separate tests was $Cov(y_{ijkl}, y_{i'jkl}) = 0.6243$. The covariance among families with one

common parent in separate tests was $Cov(y_{ijkl},y_{i'jkl'}) = 0.2930$. The covariance among families in the same test with one common parent was $Cov(y_{ijkl},y_{ijkl'}) = 0.3337$. These covariances comprise the off-diagonal elements of the V matrix. The covariance between the observed family mean and the true breeding value was $Cov(y_{ijkl},g) = 0.5860$. This last covariance is the single constant element comprising the C matrix.

The next step was to use PROC MEANS to obtain means for each family at each location. These data were modified by obtaining the differences between the family means and each location mean. These marginal means form the y vector shown in equation 1.

All of the above matrices and vectors may be manually loaded into the format appropriate for running SAS Interactive Matrix Language (IML), but for large data sets it was essential to devise a runstream which loaded these matrices automatically. The language needed to write such programs is defined in the SAS IML Guide for Personal Computers (1985). Such programs were used to load all matrices used in these analyses. Portions of the V and C matrices used in these analyses are presented in Tables 1 and 2.

Table 1. A portion of the V matrix derived from the combined ANOVA of the loblolly pine full-sib progeny test.

V={1.0088	.6243	.6243	.3337	.3337	.3337	.3337	.3337	.3337	.3337	.
.6243	1.0088	.6243	.3337	.3337	.3337	.3337	.3337	.3337	.3337	.
.6243	.6243	1.0088	.3337	.3337	.3337	.3337	.3337	.3337	.3337	.
.3337	.6243	.6243	1.0088	.2930	.2930	.3337	.3337	.3337	.3337	.
.	.	.	.	.	.	.	.	.	.	.
.3337	.3337	.3337	.3337	.3337	.6243	.6243	.6243	.6243	1.0088};	

Since the V matrix shown in Table 1 represents a single diallel crossing group, it does not indicate the full size and complexity of the full data set. The largest matrix was a 50 x 50 element matrix, the smallest a 10 x 10 element matrix. A full matrix comprising all family x location means would have required a 152 x 152 element matrix. The largest that our Compaq 386 PC could invert in the IML procedure was a 65 x 65 element matrix. The subdivision into seven unrelated crossing groups made it possible to construct matrix subsets with no loss of information.

Heterogeneous Second Moments

The procedure utilizing heterogeneous second moments requires an additional step. In this example separate sets of breeding values were predicted, each set targeted for the environment represented by one of the test locations sampled. First, ANOVAs were performed to obtain variances of family means for each location. Second, two kinds of correlations were obtained between all locations: (1) correlations based on two common parents (full-sib family means); and (2) correlations based on one common parent (half-sib family means). The variances of family means and correlations were then combined to obtain family mean covariances (Type B family mean covariances, Burdon 1977; White and Hodge 1989) among all test locations according to the following general formula:

$$Cov(y_{ijk},y_{i'jk}) = r_{Bf} [Var(y_{ijk}) Var(y_{i'jk})]^{1/2} \quad (2)$$

where $Cov(y_{ijk},y_{i'jk})$ is the Type B covariance of family means, r_{Bf} is the correlation between family means in each test, and $Var(y_{ijk})$ and $Var(y_{i'jk})$ are the variances of family means of each of the two tests respectively.

Table 2. A portion of the C matrix derived from the combined ANOVA of the loblolly pine full-sib progeny test.

C={.5860	0	0	0	.5860	0,
.5860	0	0	0	.5860	0,
.5860	0	0	0	.5860	0,
.5860	0	0	.5860	0	0,
0	0	0	.5860	0	0,
.	.	.	.	.	.,
0	0	0	0	0	.5860};

A subset of the V matrix comprising some of the heterogeneous variances and covariances produced by this approach is presented in Table 3, and a subset of the C matrix comprising the appropriate covariances is presented in Table 4.

Table 3. A portion of the V matrix derived from separate ANOVAs of loblolly pine full-sib progeny tests and Type B correlations and covariances.

V={1.0485	.7600	.5282	.4171	.5110	1.0019	.5430	.4155	1.0019	.5430 .
.7600	1.3308	.9553	.4830	.2216	.5430	.8021	.7709	.5430	.8021 .
.5282	.9553	1.5112	.6765	.0442	.4155	.7709	1.4217	.4155	.7709 .
.4177	.4830	.6765	1.1012	.5419	.7748	.9626	.9796	.4171	.4830 .
.	.	.	.	.	.	.	.	.	. .

Table 4. A portion of the C matrix derived from separate ANOVAS and Type B correlations and covariances.

C={.8342	1.0220	2.0038	1.0282	.8310	0	0	0	0 .
.9660	.4432	1.0282	1.6042	1.5418	0	0	0	0 .
1.0492	.5366	.8342	.9660	1.3529	0	0	0	0 .
.5366	.8402	1.0220	.4432	.0883	0	0	0	0 .
.8342	1.0220	2.0038	1.0282	.8310	0	0	0	0 .
.	.	.	.	.	.	.	.	. .

The following equation was used to obtain the elements of the C matrix from family mean correlations and variances of family means:

$$Cov(y_{ijk},g) = 2\, r_{Bf}(h_i,h_T) [Var(y_{ijk}) Var(y_{Tjk})]^{1/2} \quad (3)$$

where $Cov(y_{ijk},g)$ is the covariance between the observed family mean and the true but unknown breeding value of one parent (say k), $r_{Bf}(h_i,h_T)$ is the correlation between the half-sib family means of the reference test

environment and those of the target environment, and $Var(y_{ijk})$ and $Var(y_{Tjk})$ are the variances of half-sib family means of the reference test environment and the target environment respectively.

RESULTS AND DISCUSSION

A sample of the breeding values obtained by the method using homogeneous second moments is shown in Table 5, and a sample of the breeding values for each of the five target environments is given in Table 6. Space does not permit a full listing of all breeding values.

The sample of 11 of the 38 breeding values generated by the method of homogeneous second moments shown in Table 5 has a good range and shows the general forest area (GFA) check lots to be below average. One measure of the precision of BLP is the estimated correlation between the true and predicted genetic values (CRGG):

$$CRGG = Corr(\hat{g},g) = \{Var(\hat{g})/Var(g)\}^{1/2} \qquad (4)$$

(White and Hodge 1989), where $Var(\hat{g})$ is the variance of the predicted breeding values and Var(g) is the variance of the true but unknown breeding values. The CRGGs for all parents in this test equaled or exceeded 0.59, and most exceeded 0.7, which suggests very good precision for this test (homogeneous second moments only).

In southern Mississippi there is no valid reason to obtain separate sets of breeding values for each target environment. We have been unable to identify any environmental variables that provide reliable criteria for labelling such environments. For example, southern Mississippi does not have significant elevational zones. However, test 070006 is on the Holly Springs District in northern Mississippi, which is a different breeding zone. Hence, the rankings of some of the breeding values sampled in Table 6 change considerably when predicted for the target environment associated with test location 070006 on the Holly Springs District. This enables us to utilize those interactions because we can identify suitable criteria which characterize a population of such sites. If we wish to breed a set of families from southern Mississippi suitable for planting in northern Mississippi, we could set aside an orchard block consisting of those families targeted for that zone. This method of BLP based on ANOVAs in separate tests and Type B correlations and covariances between tests offers a useful and powerful tool for analyzing unbalanced data from full-sib progeny tests when target environments can be identified.

CONCLUSIONS

The precision and significance of these tests are not measurable, since we do not have the luxury of F tests or t tests. However, there are valid reasons to have some confidence in the relative precision and reliability of predicted breeding values for both methods. For example, employing the method utilizing homogeneous second moments gave high estimated correlations between the true and predicted breeding values (CRGG), on the order of 0.6 or

greater, which suggests that precise estimates of the second moments were obtained. White and Hodge (1989) note that it is probably necessary to analyze tests having at least 30 unrelated parents to obtain precise estimates of the second moments. The present set of tests would seem to satisfy this condition.

Table 5. Eleven breeding values for height at age 5 in loblolly pine for tests 070001, 070002, 070004, 070005 and 070006 obtained from best linear prediction based on homogeneous second moments.

Crossing group	Parent	Rank	Breeding value, height	Breeding value + mean height
			feet	feet
2	213	1	1.23	16.62
7	238	2	1.16	16.55
1	206	3	1.04	16.43
41	903105 1/	4	1.03	16.42
7	245	5	1.02	16.41
.	.	.	.	.
50	903413 2/	33	-0.93	14.46
8	247	34	-0.97	14.41
8	227	35	-1.23	14.16
8	243	36	-1.23	14.16
3	222	37	-1.25	14.14
3	209	38	-2 24	13.15

1/ An open-pollinated seed orchard clone. 2/ A general forest area check lot.

However, the poor sampling of the crosses resulting from the incompleteness of some of the crossing groups probably causes the predictions of those particular breeding values to be very unreliable. For example, in Table 5 parents 227 and 243 in crossing group 8 have identical breeding values because they are crossed only with each other. Decisions on the fate of parents in such poorly sampled crossing groups may be postponed until data from other tests provide more complete information.

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Table 6. Six breeding values for height of loblolly pine at age 5 for each of five target environments based on data measured in tests 070001, 070002, 070004, 070005 and 070006 obtained from best linear predictions utilizing heterogeneous second moments, including rankings of each breeding value in each test.

Crossing group	Parent	Test	Rank	Breeding value, height	Breeding value + mean height
				feet	feet
2	213	070001	4	1.53	16.92
7	238	070001	1	3.16	18.55
41	903105	070001	13	0.84	16.23
50	903413	070001	32	-2.05	13.34
8	247	070001	24	-0.76	14.63
3	209	070001	36	-2.68	12.71
2	213	070002	6	2.06	17.45
7	238	070002	1	5.92	21.31
41	903105	070002	7	1.84	17.23
50	903413	070002	33	-3.45	11.94
8	247	070002	32	-1.85	13.54
3	209	070002	15	0.16	15.55
2	213	070004	8	2.56	17.95
7	238	070004	1	5.21	20.60
41	903105	070004	9	1.68	17.08
50	903413	070004	35	-4.66	10.73
8	247	070004	34	-2.14	13.25
3	209	070004	25	-1.20	14.19
2	213	070005	8	1.29	16.68
7	238	070005	2	2.41	17.80
41	903105	070005	3	2.14	17.53
50	903413	070005	32	-1.58	13.81
8	247	070005	25	-0.57	14.82
3	209	070005	34	-2.35	13.04
2	213	070006	16	0.61	16.00
7	238	070006	31	-1.05	15.87
41	903105	070006	12	1.38	16.77
50	903413	070006	25	-0.21	15.18
8	247	070006	17	0.58	15.97
3	209	070006	37	-4.89	10.50

CONCURRENT SESSION: 3B

GALL TYPES FOR PREDICTING FUSIFORM RUST DAMAGE TO LOBLOLLY PINE IN THE FIELD

C. H. Walkinshaw[1]

Abstract.--A number of rust symptoms and their interrelationships were observed on loblolly pines in 8 locations. In one planting where infection was 90%, rust-associated mortality was 18% at age 12 and its relationship to various rust symptoms could be tested. None of the symptoms observed were highly correlated to rust-associated mortality for individual control-pollinated families. However, the number of galls growing from branches into stems in commercial plantations with 84% and 87% infection was highly correlated to rust-associated mortality. The percentage of trees with damaging galls that had moved into the stem was 83% for some of the more resistant families even though rust infection was less than 50%. The number of BG+S galls in loblolly pines is the most useful symptom to read.

Keywords: Cronartium quercuum f. sp. fusiforme, disease severity, pathogenic variation, Pinus taeda L.

INTRODUCTION

Loblolly pines (Pinus taeda L.) can tolerate rust infection (Goddard and Wells 1977, Geron and Hafley 1988), unless they are infected in the terminal shoot during their first few growing seasons, and they will often survive with rust for decades (Webb and Patterson 1984). Infected loblolly pines from different pine families vary in mortality rates, but prediction of the level of this rust-related death is difficult.

Previous studies, such as those of Geron and Hafley (1988) and Lloyd (1982), have used age, location, site index, measurements of tree growth, number of trees galled, number of galls per tree, and location of galls as possible predictors of rust-associated mortality in loblolly pine. However, death of individual rust-infected loblolly pines is not understood.

In this study, 12-year old loblolly pines with severe rust infection but with low early mortality from true stem galls were selected for describing gall types to correlate with rust damage.

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The objective was to predict mortality and stem damage in loblolly pine families. If the observed symptoms correlated with rust damage, they could be useful for monitoring rust impact in plantations. Moreover, symptoms that predict stem damage or mortality could then be incorporated into growth and yield equations.

METHODS

Definition of Symptoms and Measurements

Symptoms observed were **stunted tree** (shorter than average for the plot); **abnormal stem** (twisted, bent, or double stem, unsuitable for poles or lumber); **galled tree** (tree with one or more fusiform rust galls); **true stem gall** (stem gall from infection of terminal shoot); **branch galls growing toward, and within 30 cm of the stem** (galls on branches that are within 30 cm of the stem which remains unswollen); **branch galls that have grown into the stem (BG+S)** (galls on branches that have grown to the stem causing it to swell); **number of BG+S galls at one whorl; height on the stem of BG+S galls; percentage of the stem circumference girdled by a gall or multiple galls; branch galls** (galls on branches 30 cm or more from the stem); **tree dead from rust** (dead tree with one or more galls in the stem); **tree dead from causes other than fusiform rust;** and **witches' broom** trees (trees without a dominant stem). **Degrade** of stems is assumed to occur when one or more galls grow into the stem from a branch infection.

Heavily Infected Planting

The Bumpnose area near Greenwood, Florida, is planted in pines 10 to 15 years old. The fusiform rust incidence is 95% in loblolly pines planted ajacent to the observation area. Susceptible slash pine nearby are 85% infected. Livingston Parish loblolly pine has 41% rust.

In the observation area, there were 49 full-sib families of loblolly pine planted in five replications of 10-tree row plots at a spacing of 10 x 10 feet. One replication had poor survival and was not used. Each tree was evaluated for fusiform rust at age 12. Trees had been previously assessed by others for mortality at age four.

Low-Rust Plantings

Progeny tests located in Mississippi on the the DeSoto National Forest near Wiggins (1 test), on the Bienville National Forest near Raleigh (2 tests), and on the Homochitto National Forest near Gloster (2 tests), were of low-rust incidence. More than 50 full-sib families of loblolly pines planted in these National Forests were observed; only 20 families were planted in more than one forest. Spacing in all three locations was 8 x 8 feet. Three to four replications of 10-tree row plots were

observed at age eight and verified at age nine.

Average infection for susceptible families was 37% for the DeSoto, 12% and 50% for the Bienville, and 26% and 30% for the Homochitto National Forests. The highest infection for any family was 63%. Only 3% of the trees in the progeny tests had died from rust at the time of observation. Of these dead trees, 33% had witches' broom.

Commercial Stands

Measurements of 300 loblolly pines were observed in commercial plantings in Holmes County, Florida, and Stone, Marion, and Pike Counties, Mississippi. Dead and living trees, 12 to 20 cm dbh, were examined for numbers of BG+S galls, branch galls growing toward the stem and within 30 cm of the stem, and branch galls located 30 cm or more from the stem. Random rows of 25 trees were observed. Observations were made during aecial sporulation to increase accuracy of measurements.

Analyses

Analyses of variance (fixed effects model) and regression analyses were done to estimate family effects and to test relationships of symptoms among each other for all observation areas.

RESULTS

Observations in a Heavily Infected Planting

At the time of observation,99% percent of the trees had fusiform rust galls. Only 2% of the trees had died from true stem galls; 38 of the 49 families had no true stem galls. Nearly all the rust-associated mortality occurred after age four. Of the 49 families, 12% of the trees died from causes other than fusiform rust (including mortality at planting). Rust-associated mortality for the families at age 12 ranged from 3 to 41% of trees (Table 1).

Infected trees averaged three BG+S galls. Such galls occurred at heights from 15 cm (6 inches) to 7.6 m (25 feet). The lowest BG+S galls averaged 91 cm (3 feet), and the highest averaged 183 cm (6 feet). When means for BG+S galls and branch galls within 30 cm of the stem were totalled, the average infected tree observed had eight galls growing towards or into the stem.

Table 1.--Fusiform rust symptoms on 12-year-old loblolly pines growing near Greenwood, Florida, for which family effects are significant[1]

Pine cross	Abnormal stems	Trees galled	Branch-to-stem galls	No. of galls[2]	Rust mortality
	%	%	%		%
3-17X1-11	3	71	54	4	17
3-17X1-14	2	83	51	4	3
3-17X1-64	8	78	44	4	5
3-17X3-2	19	86	66	5	5
3-17X3-7	17	76	56	5	5
3-17X3-8	20	92	72	6	8
3-2X1-11	12	95	79	5	18
3-2X11-20	8	92	80	6	27
3-2X1-14	8	88	89	6	28
3-2X12-12	7	100	82	7	23
3-2X12-13	12	94	95	4	22
3-2X1-64	5	90	84	4	26
3-2X5-33	6	98	81	5	34
3-2X5-5	0	91	71	7	28
3-2X7-2	8	97	97	6	21
3-2X7-34	6	97	97	6	24
3-2X7-56	19	95	74	7	16
3-34X1-11	3	100	96	6	39
3-34X1-14	12	94	86	7	22
3-34X3-2	8	100	100	4	26
3-36X1-11	15	90	75	4	8
3-36X11-20	10	78	65	4	11
3-36X1-14	4	87	68	4	17
3-36X12-12	0	78	66	4	8
3-36X12-13	10	92	86	4	24
3-36X1-64	16	77	61	4	15
3-36X3-17	0	92	64	2	10
3-36X5-33	10	77	56	3	6
3-36X5-5	5	92	82	6	10
3-36X7-2	20	81	66	3	11
3-36X7-34	6	83	58	3	16
3-36X7-56	8	86	82	3	5
3-8X1-11	28	92	85	8	13
3-8X11-20	13	59	45	5	14
3-8X1-14	10	94	74	4	14
3-8X12-12	19	97	97	5	28
3-8X12-13	25	100	84	6	20
3-8X1-64	18	90	76	5	31
3-8X5-33	8	97	94	4	6
3-8X5-5	17	100	83	6	10
3-8X7-2	18	89	82	6	6
3-8X7-34	13	90	80	8	18
3-8X7-56	2	92	88	7	10
10-37X3-2	7	94	86	6	33
10-37X3-36	7	94	79	4	18
10-37X3-8	14	98	92	10	23
10-39X10-8	9	100	96	9	26
11-23X10-18	0	96	96	6	41
10-8XW	10	100	91	6	18

[1]Means of 4 replications of 10 trees each; see text for description of symptoms.

[2]Average number of branch galls into the stem and within 30 cm of the stem.

Family effects were significant for five symptoms (Table 1). Signicance probabilities ranged from 0.0094 to 0.0001; replication effects did not differ significantly. Percentage of trees galled explained 71% of the variability of BG+S galls. Neither BG+S galls nor percentage of trees galled explained variation in rust-associated mortality (r^2 =32% and 21%). When percentages of BG+S galls and trees with abnormal stems were combined they accounted for only 37% of the variation in rust associated mortality. Galled trees that died had 4 to 15 BG+S galls per tree; 31% of these trees had 10 or more BG+S galls.

The mean across families and replications for the percentage of tree circumference composed of gall tissue was 55%. Differences in girdling for families and replications were significant (P=0.05). Family 3-34 x 1-11 had the highest mean at 68%; family 3-17 x 1-11 had the lowest at 43%. Variability in stems girdled by gall tissue was not explained by BG+S galls (r^2=4.7%).

Branch galls more than 30 cm from the stem occurred on 62% of the trees. Means for the 49 families ranged from 42% to 81%. Family and replication effects were significant (P=0.05). Incidence of branch galls 30 cm or more from the stem accounted for 40% of the variation in percentage of trees galled and 22% of the variation in percentage of BG+S galls.

Observations in Low-Rust Plantings

Families differed significantly (P=0.05) within each test for percentage of trees galled and percentage of BG+S galls in the low-rust plantings. Percentage of galls within 30 cm of the stem was low for all families (Table 2); crosses with 209 as the female parent had low percentage of trees galled and few BG+S galls.

Multiple regression analyses did not reveal relationships among symptoms. For example, regression of the family means did not show any relationship between abnormal stems and other symptoms. Only 10% of the variation in percentage of trees galled could be accounted for by other symptoms.

When incidence of gall types for five of the families in Table 2 were compared at Bienville and Homochitto Forests, location had little effect on occurrence of BG+S galls. Family 250 x 238 had means of 70% and 71% for BG+S galls at the two locations, and family 236 x 228 had 86% and 73% BG+S galls. Family 203 x 233 had the highest percentage of branch galls at both locations; however, BG+S galls were correspondingly low.

Table 2. Percentage of rust symptoms on Forest Service loblolly pines growing in southern and central Mississippi.[1]

Pine cross	Abnormal stem	Trees galled	Branch to stem galls	Branch galls within 30 cm of stem	Branch gall more than 30 cm from stem
	. . . . . %				
203X223	2	43	26	2	89
206X231	9	58	48	7	56
209X203	6	4	0	0	100
209X221	6	2	0	0	0
209X239	16	6	40	0	60
213X202	21	23	57	0	36
216X238	4	16	47	13	47
216X245	4	22	83	8	25
217X247	20	36	28	6	44
222X239	12	20	60	0	60
223X221	4	30	25	25	69
236X228	8	35	59	7	48
236X231	11	36	45	4	68
243X227	0	22	67	5	52
250X208	7	30	43	3	54
250X238	6	23	52	8	48
250X242	7	14	0	0	100
250X245	4	20	25	8	75
3-3-2	8	34	36	13	63
3-4-2	16	27	65	14	42

[1]Means of 2 to 5 locations and 3 to 4 replications of 10 trees per family. Trees were read at 8 and 9 years after planting.

Observations on BG+S in Commercial Stands

Results from high and low rust plantings suggested that variability of rust mortality might be explained by the number of BG+S galls. To test this hypothesis, galls were observed in commercial loblolly pines that had 60%, 67%, 84%, and 87% infection (Stone, Pike, Holmes, and Marion counties). Rust-associated mortality was 0%, 6%, 12%, and 20% for the four plantings. As shown in Figure 1, variation in this mortality is explained to a large degree by the number of BG+S galls per tree (r^2 =85% and 87% for sites with 84% and 87% infection).

Of the infected trees on the four sites, 87% had BG+S galls. Only 12% of the trees had branch galls and branch galls growing toward the stem but not into the stem. The number of trees with one BG+S gall ranged from 71 to 75 per location. Rust-associated mortality was not observed in trees with one BG+S gall. Moreover, rust mortality was not seen in trees with two BG+S

galls except on the site with 8% infection.

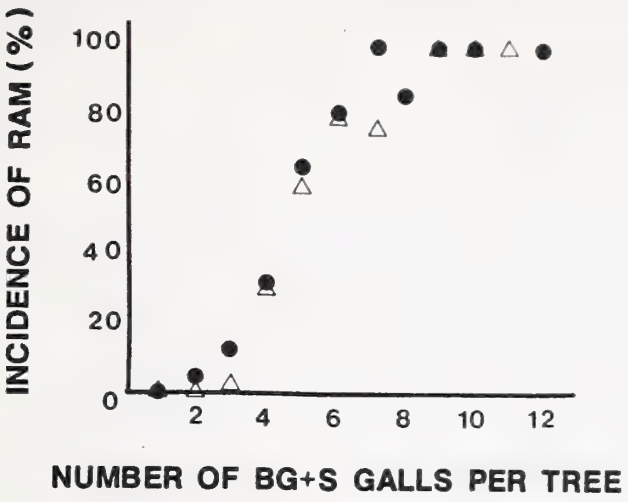

Figure 1. Relationship between rust-associated mortality (RAM,%) and BG+S galls in Mississippi (circles) and Florida (triangles) commercial loblolly pine.

DISCUSSION

The close relationship between high incidence of rust, BG+S galls, and mortality in commercial plantings was unexpected from analysis of family data in Table 1. However, if crosses with the same female (Table 1) are averaged, mortality, galled trees, and BG+S galls (not shown in Table 1) appear related.

Rust-associated mortality in individual families in the full-sib Florida plantation appeared unrelated to symptoms that describe fusiform rust-infected trees at age 12 (Table 1). Had equal numbers of crosses with female 3-17 been tested with females 3-2 or 3-34, the percentage of trees with galls and BG+S galls would have been related to mortality. The mean of 12 crosses with female 3-36 was 12% RAM while the mean for 11 crosses with female 3-2 was 24% RAM. The values for BG+S galls were 69% and 81% for the means of the crosses.

BG+S galls occurred in high percentages in the full-sib plantations studied in Florida and Mississippi. These galls severly damaged the stem and caused degrade, especially when they entered the stem at the heights in the Florida planting. These trees would not be suitable for high value forest products. While occurrence of BG+S galls was correlated to other gall types in the Florida planting, infection in Mississippi forests was too low for correlating symptoms.

Although percentage of abnormal stems in the Florida planting was significant for families, it did not relate to BG+S or other symptoms. Comparison of families 3-17 x 3-8 and 3-2 x 5-5 shows that while incidence of abnormal stems differed by 20%, these families had nearly identical values for trees with galls and BG+S galls (Table 1). Loss of apical dominance (abnormal stems) will be lethal for such trees after crown closure.

Most trees in the full-sib Florida test with BG+S galls had straight stems after repeated invasions, possibly because healthy tissue had formed by the time the fungus entered the stem. Although some trees with BG+S galls had gall tissue in 50% to 90% of their circumferences, gall tissue overlay 4- to 10-year-old normal stem tissues. Thus, invasion of the stem from branches was restricted to part of the trees cross section.

Breeding for resistance should exclude loblolly pine parents that form BG+S galls in large numbers. Crosses 209 x 203 and 250 x 242 might be desirable selections because infections generally occurred more than 30 cm distal to the stem (Table 2). Infected trees of a potentially poor candidate, cross 216 x 245 formed BG+S galls on 83% of the stems. One should examine growth habits of crosses such as 216 x 245 and choose families whose branches elongate rapidly. The highly susceptible Family 203 x 223 and the resistant pine 209 crosses seem to be of this type. Moreover, other studies suggest that selection of trees that form round rather than elongated galls should exhibit fewer BG+S galls (G. A. Snow, personal communication). The high incidence of BG+S galls in infected Mississippi forests shows that degrade may be significant even though the percentage of galled trees is relatively low.

CONCLUSIONS

The number of BG+S galls in loblolly pines is the most useful symptom; it classifies a tree as susceptible either to degrade or rust-associated mortality. Ultimately, BG+S gall data might be incorporated into growth and yield models and recommendations for thinning.

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FIELD PERFORMANCE OF SLASH PINE FROM FUSIFORM RUST-RESISTANT FAMILIES

R. C. Froelich[1]

Abstract.--Six families of putatively fusiform-rust resistant slash pine (Pinus elliottii Engelm. var. elliottii) were planted in four consecutive years at three locations in south Mississippi, and fusiform rust infections were documented according to the calendar year in which they occurred. Each of 24 plots contained 80 trees--13 or 14 established trees of each family in a completely random arrangement. Rankings of the six families tested were highly variable in different trials that separated infection by year of occurrence. Every family ranked well in some trials and poorly in other trials. Much of the variation was apparently due to the low number of trees for computing percentage with infection, but there was some evidence to confirm that relative family performance was probably influenced by the local (plot) environment as well as by differences in the environment among locations. Although there were considerable variations in relative family performance among trials, three or four families performed relatively well with respect to rust infection in a majority of the trials at all locations while two families usually did poorly. Replicating experiments over time on individual locations and separating infection by year of occurrence provides added dimension to field experiments that evaluate relative performance of pine families to fusiform rust.

Keywords: Cronartium quercuum f. sp. fusiforme, family plots, Pinus elliottii, rust-resistance

INTRODUCTION

When families of pine bred for resistance to fusiform rust caused by the fungus Cronartium quercuum f. sp. fusiforme are planted at different locations, there often seems to be considerable variation in relative rust infection among locations. Some of this variation may be due to differences in the male parent since test materials are usually open pollinated, and seed collections may be made in different years or in different orchards developed from common ortets. But even when plantings are derived from common seed lots there still seems to be considerable variation in relative performance among locations (Goddard and Schmidt 1979, Wells and Wakeley 1966). My purpose in this paper is to examine variations in infection that occurred when common seed lots of six putatively rust-resistant slash pine (Pinus elliottii Engelm. var. elliottii) families were planted in four consecutive years at three

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locations in south Mississippi and examined for infection occurring in consecutive growing seasons.

METHODS

The data for studying variation in fusiform rust infection were available from a large study in south Mississippi (Froelich, 1988) to evaluate effects of fusiform rust on growth and yield of slash pine and loblolly pine (P. taeda L.). Rust infection of susceptible seed lots of slash and loblolly pine, and resistant loblolly pine is given in Froelich (in press). The current study focuses on twenty-four 80-tree plots of putatively rust-resistant slash pine (three locations in Harrison or Hancock counties x four consecutive annual plantings at each location x two replications per year in each annual planting). The rust infection data for the 24 plots are unique in several respects:

(1) The four consecutive annual plantings at each location were developed from common seed lots.

(2) Each plot of 80 trees contained six putatively resistant families (six seed lots), which were randomly planted instead of planted in row plots.

(3) All galls were tallied and year of gall origin was identified, making it possible to compute percent infection by calendar year of occurrence.

The six families were provided by forest industries who considered them to have high degrees of resistance to fusiform rust. The seed lots were obtained from wind-pollinated trees in an orchard developed for resistance. Previous seed collections from these families apparently had developed less rust in field and greenhouse trials. In this study, however, the six families collectively developed only slightly less rust than a seed lot obtained from an orchard that had not been developed for resistance to rust.

Uniform establishment density of 80 trees per plot was ensured by hand planting two trees about 6 inches apart (6-ft x 10-ft intervals) and removing one when necessary at the beginning of the second growing season. Subsequent mortality, almost all rust-related, reduced numbers of trees available for computing percentage of infection, but the number of trees usually exceeded 10 trees of each family per plot even after the fifth growing season. This low number of 10 to 13 trees per plot will be shown to be marginal for estimating family performance. Percentage of infection was computed as the number of trees that developed one or more galls (branch gall or stem gall) in a specified growing season divided by the number of trees living at the end of the growing season. Data from only the two most heavily rust-infected plantings are given in this report. These 2 plantings were about 13 miles apart and the 16 plots of putatively resistant slash pines were of similar site quality; average heights of dominant and co-dominant trees ranged from about 21 to 25 feet per plot at age seven. Annual infection in plots at the third location rarely exceeded 30%, but the data from this location supported the conclusions derived from the two other plantings.

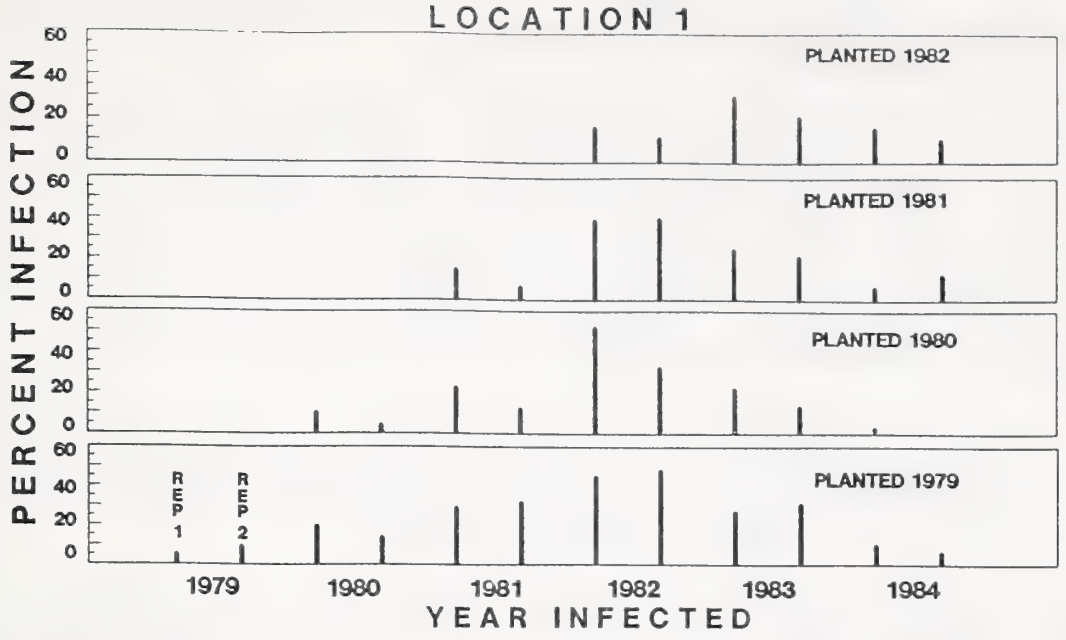

Figure 1a. Consecutive annual fusiform rust infection in 8, 80-tree plots of putatively rust-resistant slash pine (four planting years x two replication plots per planting year) at Location 1. Pairs of bars indicate Replications 1 and 2.

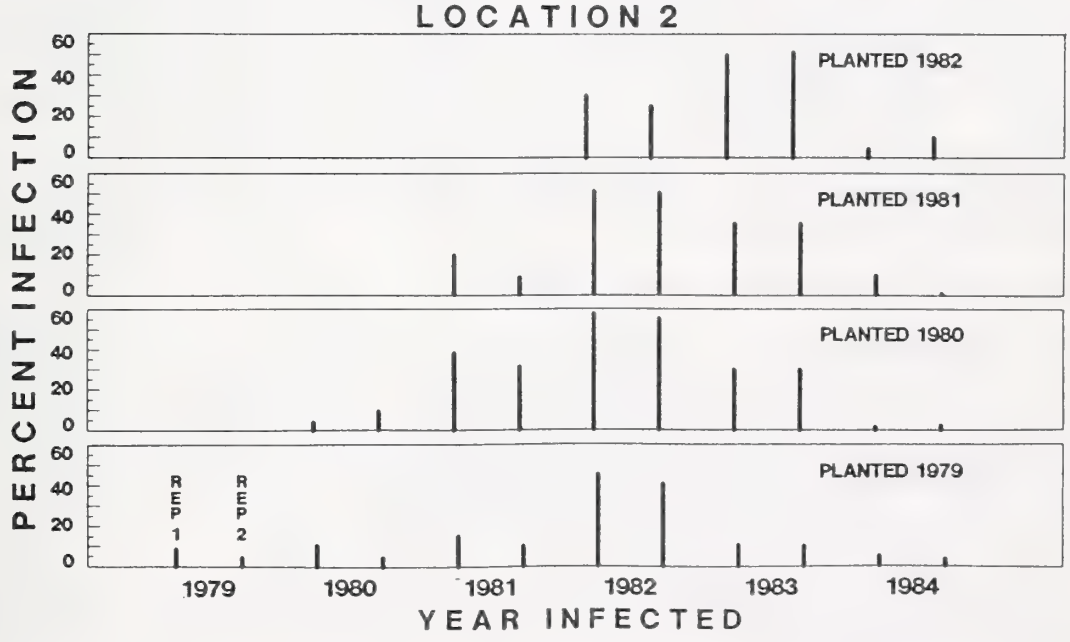

Figure 1b. Consecutive annual fusiform rust infection in 8, 80-tree plots of putatively rust-resistant slash pine (four planting years x two replication plots per planting year) at Location 2. Pairs of bars indicate Replications 1 and 2.

RESULTS

Variation in Rust Infection Among 80-Tree Plots.

Variation in annual rust infection in the two plantations developing the most rust is shown in Figures 1a and 1b. These combined data for all six families show that the largest variation in infection between identical 80-tree plots (replications) was 20% (Location 1, year planted = 1980, year infected = 1982). These two plots were located about 150 feet apart. The largest variation in annual infection noted among replications of putatively nonresistant slash pine (not shown in this report) was 24% (range of 16 to 40% among four identical plots). However, the usual variation in infection between replications was less than 10% in both resistant and nonresistant slash pine.

Combined family data indicated considerable variation in infection from year to year in individual plots. In most plots the peak year for infection was 1982. However, in four plots established in 1982 (two each in Locations 1 and 2), most infection occurred in 1983 (Figures 2a, 2b).

All the 80-tree family plot data also showed that infection varied by age class. In general, very little infection developed in the first growing season; this may be the reason why infection was greater in 1983 than in 1982 in some plots. A maximum of 25 to 30% infection was noted in the first growing season (planted and infected in 1982) at Location 2 (Figure 1b). This was about one-half the infection noted in 1982 for trees that had been planted one year earlier and were in their second season of growth in 1982. After the first growing season, age-class-related patterns of infection could be observed. For example, in 1983 (Location 2), about 50% infection developed in the second-year age class. The third- and fourth-year age classes ranged from 30 to 35% infection and in the fifth-year age class only 10% infection was observed. Similar age-class-related patterns were not observed in 1983 at Location 1. However, the collective evidence from both resistant and nonresistant plots of slash pine in three locations indicated that probabilities for slash pine infection decreased dramatically after about the fourth or fifth growing seasons.

Performance of Individual Slash Pine Families.

The individual family breakdown of 80-tree plot infection data is shown in Figures 2a and 2b. These data include only the infection years for which at least one family developed 40% or more infection in one of the two replication plots. There is considerable variation in relative rust infection among 34 trials--14 trials at Location 1 (Figure 2a) and 20 trials at Location 2 (Figure 2b)[2]. Every family can be found at the top or bottom of the infection scale in at least one trial. Much of this variation is apparently related to inadequate sample size for computing percentage of infection. Still, several observations merit consideration. Families 1 and 3, for example, commonly

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[2]Each trial shows rust infection by plot and family for individual calendar years.

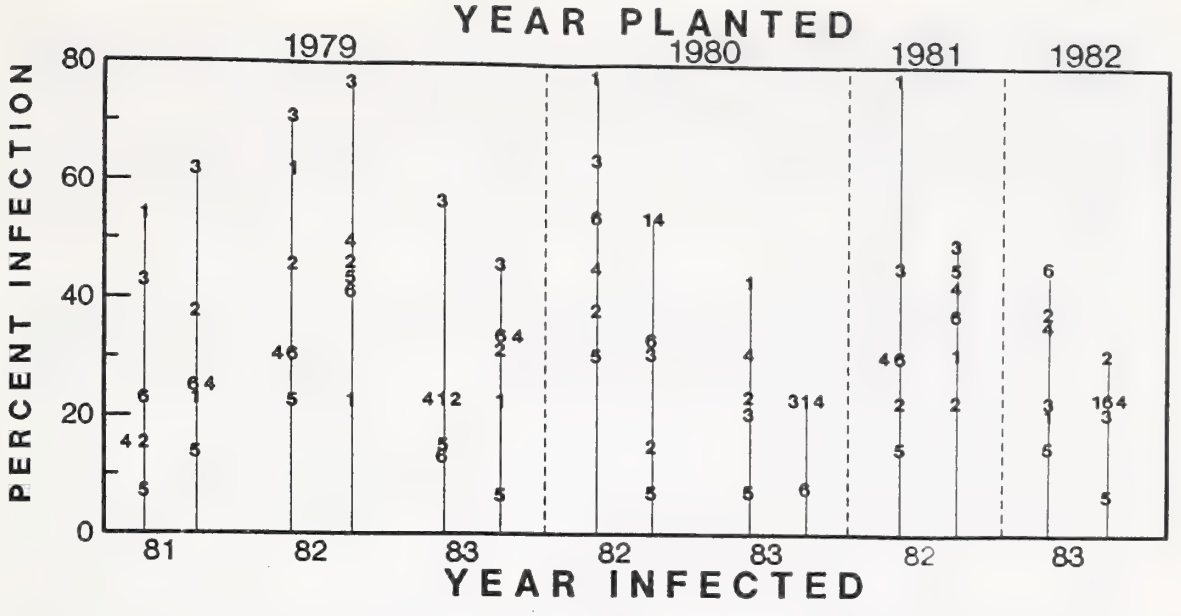

Figure 2a. Annual fusiform rust infection of six putatively rust-resistant families of slash pine at Location 1. Numbers are family identification. Missing data are zero. Pairs of bars indicate Replications 1 and 2.

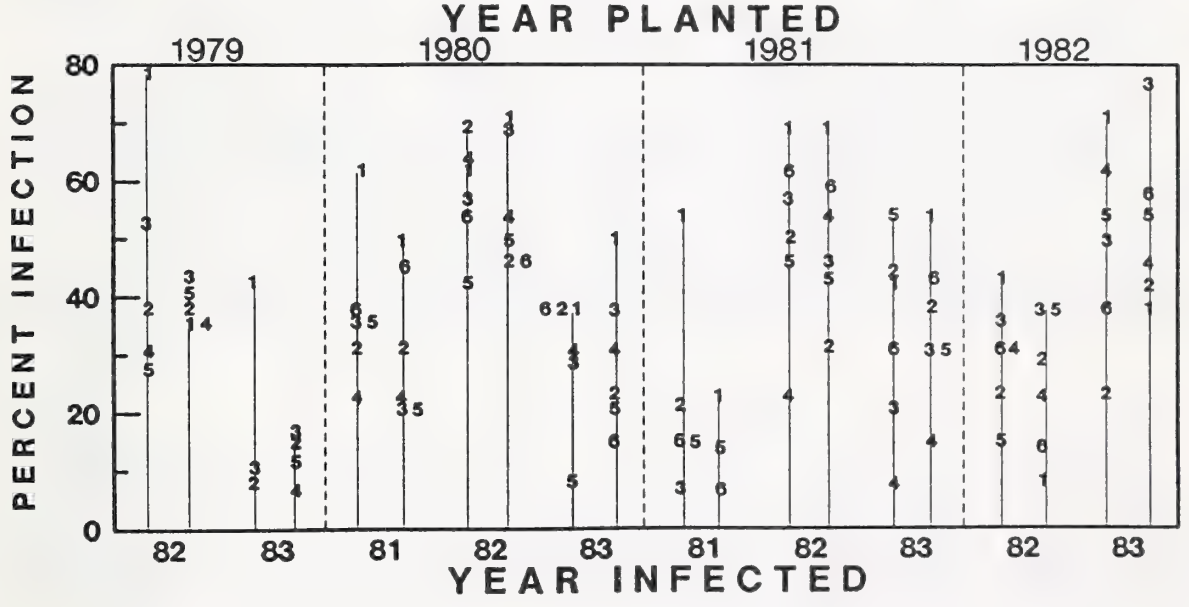

Figure 2b. Annual fusiform rust infection of six putatively rust-resistant families of slash pine at Location 2. Numbers are family identification. Missing data are zero except for family 6 which survived poorly in the 1979 planting and is not presented. Pairs of bars indicate Replications 1 and 2.

developed the most infection. What may be of most importance is that neither of these two apparently most susceptible families occurs at the bottom of the infection range in both replications of the individual trial pairs. Similarly, the four most resistant families seldom occur at the top of the infection range in both replications of trial pairs. With sufficient replication, therefore, it still appears possible to determine relative performance even when sample size is minimal and relative rankings are highly variable among trials.

Although statistical analysis did not seem appropriate because of the marginal sample of trees, several observations may be important relative to possibilities of family x location interactions. To illustrate, in the 1979 planting at Location 1 (Figure 2a), Family 3 developed more rust than Family 1 in five of six trials (exception Replication 1, 1981); the differences in percentage of infection between the two families were quite large in four of the trials. In the 1980 plantings at Location 2 (Figure 2b), however, the pattern was reversed; there was more infection from year to year in Family 1 in all six trials. Another interesting comparison is the 1983 infection of Families 1, 2, and 3 in the 1982 planting at Location 1. Families 1 and 3 developed intermediate levels of infection in both replications; while Family 2, usually with very light infection, developed relatively high infection in both replications.

A final observation suggesting possibility of family x location interaction is that Family 5 emerges clearly as most resistant in Location 1; it was at or near the bottom of the infection range in virtually every trial. At Location 2, however, this family occupied an intermediate position on the infection scale in half of the trials.

Although cumulative infection data are not given, many of the same observations or impressions would have been obtained if only these data had been available. For example, relative cumulative infection of families was just as variable as was the annual infection data. Families 1 and 3 frequently developed the most cumulative infection and Family 5 developed relatively lower infection at Location 1 than at Location 2. The main advantage of separating infection by year of occurrence, therefore, seems to be the added information it gives to field experiments.

DISCUSSION

Although sample size (number of trees of each family per plot and number of replications per annual planting) was too small to permit definite conclusions, there was fairly strong circumstantial evidence to indicate that relative rust infection of six slash pine families was affected by variations in environmental conditions within and between plantings. From the variations in infection observed in various plantings, it is easy to visualize that significant location x family interactions are likely to occur in some field experiments. However, by replicating plantings in time with the same seed lots on each site, and documenting infection by annual year of occurrence, the various interactions seemed to take on questionable practical significance as suggested by Goddard and Schmidt (1979). Specifically, there was no overwhelming consistency to the interactions: one family may have been more

suggested by Goddard and Schmidt (1979). Specifically, there was no overwhelming consistency to the interactions: one family may have been more heavily infected from year to year in plantings established in one calendar year, but in plantings established in another year on the same site, that same family may have developed relatively less infection in consecutive growing seasons.

More comprehensive testing would be needed to determine whether location x family interactions are an important biological phenomenon to be given serious consideration by tree breeders, or are more of an artifact associated with inadequate study design, lack of replication, or insufficient numbers of trees of each family per plot. Future field testing might take into account the following suggestions:

(1) Studies of relative family performance in field trials would be more definitive if they were replicated in time on sites high in hazard to fusiform rust, using trees derived from the same seed lots in all consecutive plantings;

(2) Large plots should be considered for evaluating family performance and all families should be randomly located (a scattered arrangement rather than a pure plot arrangement) within each plot to help ensure that family performance is not confounded by variations in the local environment. Experiment size and plot size would be governed by number of families to be evaluated, but there probably should be a minimum of perhaps 30 established trees of each family per plot; and

(3) Infection data could be recorded by year of occurrence for about the first five growing seasons instead of as cumulative infection. Some added costs are involved in obtaining these data, however, because annual height measurements are essential for accurately classifying infection by year of occurrence.

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FUSIFORM RUST IN HALF-DIALLEL CROSS PROGENIES OF RESISTANT OR SUSCEPTIBLE LOBLOLLY AND SLASH PINES[1/]

E. R. Sluder[2/]

ABSTRACT. Progenies from a half-diallel cross among six loblolly pines and another among six slash pines were field-tested in central Georgia for fusiform-rust resistance. Three of each set of six parents had been typed as resistant (R) and three as susceptible (S) to the fungus in previous progeny tests that included check lots. At age 5 years, the 15 progenies of each species varied significantly in percentage infection and number of galls per tree. The RXR slash crosses produced highly resistant offspring but the RXR loblolly crosses produced progenies that were 79 percent infected. All SXS crosses of both species produced very susceptible progenies and RXS progenies were intermediate in susceptibility. Each set of parents varied significantly in general and specific combining abilities. The mean breeding values for the R slash parents were 25.5 for percent infection and 0.57 for galls per tree. For loblolly R parents, the corresponding values were 79.4 and 3.05. For the S parents, the respective breeding values were 94.1 and 3.26 for slash and 96.1 and 6.43 for loblolly.

Keywords: Pinus taeda, Pinus elliottii, Cronartium quercuum f. sp. fusiforme.

The fusiform-rust fungus (Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme) seriously decreases wood production in plantations of loblolly Pinus taeda L.) and slash (P. elliottii Engelm. var. elliottii) pines in much of the Southeastern U. S. (Anderson and others 1986). Losses occur as mortality, decreased growth of infected stems, and altered wood properties and decay in cankered tissue (Sluder 1977a). Breeding for genetic resistance to the fungus is considered the most practical way to reduce losses in commercial plantations of these two pine species. Both research on and applied breeding programs for rust resistance in slash and loblolly pines are being conducted. This paper reports the rust resistance in a half-diallel cross among six loblolly pine clones and another among six slash pine clones. In each species, half of the clones were typed as resistant and half were typed as susceptible to rust.

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MATERIAL AND METHODS

The parents in this study were seed orchard clones that had been progeny tested for resistance to fusiform rust. Six loblolly and six slash pine clones were selected for study. In field tests of each set of six clones, progenies of three had proven more resistant and three less resistant than the check lot to fusiform rust (Table 1).

The six clones of each species were crossed in a modified half-diallel (no selfs) arranged according to flowering phenology (Sluder 1977b). Seedlings from the crosses were field-planted in 1978 in Houston County, Georgia, in an area of known high incidence of fusiform rust. Plantings included four replications in randomized complete blocks. Each replicate had a 16-tree plot of each progeny. Each plot had two rows of 8 trees spaced 2.5 m between and 1.25 m within rows. Study sites for the two species were adjacent.

At age 5 years the number of galls on each tree was recorded and the percentage of the trees infected in each plot was calculated. Analyses of variance and diallel analyses (Model I) were performed on the data for each half-diallel. From the diallel analyses, variance components were calculated and used to estimate heritabilities of percentage infection and galls per tree at age 5 years (Becker 1984). Breeding values of each clone were calculated for these two traits.

RESULTS

For each species, the progenies varied significantly both in percentage infection and galls per tree (Table 2). The resistant x resistant (RXR) and the resistant x susceptible (RXS) crosses of slash pine had less rust than did the same types of crosses of loblolly pine. The SXS crosses were very susceptible in both species. Relative susceptibility of the three types of crosses was RXR<RXS<SXS for each species (table 2).

Table 1.--Rust infection and galls per tree on progenies of loblolly and slash pine parents in previous progeny tests (percent differences from check lots).

Parent	Rust rating[1/]	Rust trait			
		% infected		Galls/tree	
		(%)[2/]			
		loblolly	slash	loblolly	slash
1	R	-2	-44	-24	-28
2	S	1	31	59	96
3	R	-10	-58	-50	-21
4	R	-39	-35	-44	-6
5	S	3	26	81	74
6	S	21	134	35	49
MEAN	R	-17	-46	-39	-18
	S	8	64	58	73

[1/] R = resistant, S = susceptible to fusiform rust.
[2/] Percent = [(progeny mean - check mean)/check mean]x100. Negative values are desirable, positive values are undesirable.

Table 2.--Mean fifth-year fusiform-rust data from half-diallel crosses among six loblolly and six slash pines.

Parent			Trait	
Female	Male		Percent Infected	Galls/Tree
			Loblolly	
4 (R)[1/]	3 (R)[1/]		62.0 a[2/]	1.67 a[2/]
5 (S)	4 (R)		76.5 b	2.56 ab
6 (S)	4 (R)		82.8 bc	3.50 a-c
3 (R)	1 (R)		84.0 b-d	3.59 a-c
5 (S)	1 (R)		87.5 b-e	6.11 c-e
2 (S)	1 (R)		88.2 b-e	4.69 b-d
6 (S)	1 (R)		89.4 c-e	5.06 b-d
4 (R)	1 (R)		90.6 c-e	4.09 a-d
6 (S)	3 (R)		91.4 c-e	4.38 b-d
6 (S)	2 (S)		92.0 c-e	5.86 cd
5 (S)	3 (R)		92.1 c-e	4.30 b-d
4 (R)	2 (S)		92.2 c-e	5.11 b-d
3 (R)	2 (S)		93.4 c-e	6.52 de
6 (S)	5 (S)		96.9 de	5.18 b-d
5 (S)	2 (S)		98.4 e	8.46 e
		Mean: RXR	78.9 a[3/]	3.12 a[3/]
		RXS	88.2 b	4.69 b
		SXS	94.0 c	5.91 c
			Slash	
4 (R)	3 (R)		18.1 a	0.49 a
3 (R)	1 (R)		28.6 ab	0.78 ab
4 (R)	1 (R)		39.2 bc	1.02 ab
6 (S)	3 (R)		42.5 bc	0.89 ab
4 (R)	2 (S)		46.5 bc	1.16 a-c
3 (R)	2 (S)		47.8 bc	1.33 a-c
5 (S)	3 (R)		52.9 cd	1.05 ab
2 (S)	1 (R)		55.6 cd	1.00 ab
6 (S)	1 (R)		58.6 cd	2.02 cd
5 (S)	4 (R)		59.2 cd	2.04 cd
5 (S)	1 (R)		70.5 d	1.70 b-d
6 (S)	4 (R)		94.1 e	3.18 ef
6 (S)	2 (S)		98.3 e	2.54 de
5 (S)	2 (S)		99.2 e	3.61 f
6 (S)	5 (S)		100.0 e	4.68 g
		Mean: RXR	28.6 a	0.76 a
		RXS	58.6 b	1.60 b
		SXS	99.2 c	3.61 c

[1/] R = rated resistant, S = rated susceptible to fusiform rust.
[2/] Within a column, means followed by a common letter do not differ at the 0.05 level, Duncan's Multiple Range Test.
[3/] Within a column, cross type means not followed by a common letter differ at the 0.05 level.

The diallel analyses of plot means revealed highly significant differences among the six parents in general combining abilities (GCA) for both traits in both species (table 3). The only instance of nonsignificant variation in specific combining ability (SCA) of the parents was for galls per tree in loblolly pine. With individual-tree data, the loblolly as well as the slash pine parents showed highly significant GCA and SCA variation in number of galls per tree (Table 3). GCA and SCA estimates based on plot means are shown for the two traits in Tables 4 (loblolly) and 5 (slash).

Table 3.--Diallel analyses of variance of fusiform-rust data from half-diallel crosses among six loblolly and among six slash pines, age 5 years.

Source of variation	Trait			
	% Infected		Galls/tree	
	Mean square			
	Plot Means[1/]			
	loblolly	slash	loblolly	slash
GCA[2/]	122.43**	1691.30**	5.65**	3.14**
SCA[3/]	58.47**	144.21*	1.12	0.50**
Error (BXC)[4/]	17.99	38.57	0.66	0.09
	Individual tree[5/]			
GCA	--	--	293.66**	88.85**
SCA	--	--	115.16**	25.04**
Error (BXC)	--	--	32.80**	5.03*
Within plot			17.46	3.52

[1/]The Model I (fixed effects) analysis was used: both the GCA and SCA mean squares were tested against the BXC error mean square.

[2/]General combining ability.

[3/]Specific combining ability.

[4/]Blocks x crosses interaction.

[5/]The GCA, SCA, and BXC mean squares were tested against the within-plot mean square (Model I).

**Significant at the 0.01 level.

*Significant at the 0.05 level.

Table 4.--General (GCA) and specific (SCA) combining abilities based on plot means at age 5 years for a half-diallel cross among six loblolly pines.

Male parents	Female parents (S) 2	(R) 3	(R) 4	(S) 5	(S) 6	GCA
	----------SCA----------					
			Percent infected			
1 (R)[1/]	-6.03	0.09	11.39	-3.53	-1.91	0.14
2 (S)		3.36	6.86	1.24	-5.43	6.27
3 (R)			-13.01	5.26	4.29	-4.06
4 (R)				-5.63	0.39	-8.76
5 (S)					2.66	3.07
6 (S)						3.34
			Galls/tree			
1 (R)	-1.75	-0.30	1.08	0.68	0.29	-0.04
2 (S)		0.85	0.32	1.25	-0.69	1.74
3 (R)			-0.57	-0.36	0.38	-0.81
4 (R)				-1.22	0.38	-1.69
5 (S)					-0.36	0.73
6 (S)						0.07

[1/] R = rated resistant, S = rated susceptible to fusiform rust.

Table 5.--General (GCA) and specific (SCA) combining abilities based on plot means at age 5 years for a half-diallel cross among six slash pines.

Male parents	Female parents (S) 2	(R) 3	(R) 4	(S) 5	(S) 6	GCA
	----------SCA----------					
			Percent infected			
1 (R)[1/]	-3.21	9.08	2.89	3.05	-11.79	-12.78
2 (S)		4.58	-13.53	7.99	4.19	10.92
3 (R)			-2.52	1.09	-12.24	-28.47
4 (R)				-9.42	22.57	-11.63
5 (S)					-2.72	19.50
6 (S)						22.47
			Galls/tree			
1 (R)	-0.29	0.76	0.17	-0.45	-0.19	-0.66
2 (S)		0.53	-0.47	0.68	-0.45	0.12
3 (R)			0.13	-0.57	-0.82	-1.16
4 (R)				-0.45	0.63	-0.32
5 (S)					0.83	0.98
6 (S)						1.04

[1/] R = rated resistant, S = rated susceptible to fusiform rust.

The family heritability for percentage of progeny infected was considerably higher for slash than for loblolly pine. Heritability of number of galls per tree was nearly the same for the two species on a family basis, but was twice as high for slash as for loblolly pine on an individual-tree basis (Table 6).

In slash pines, the R and S parents differed greatly in breeding values. The R parents, when crossed with each other or trees of equal genetic value, are expected to produce offspring which would average only about 25 percent infection when planted on high-hazard sites (Table 7). Offspring from the S slash parents would average about 94 percent infection under similar conditions. Breeding values for galls per tree showed a similar contrast between R and S slash pine parents.

Table 6.--Heritabilities of rust traits at age 5 years for slash and loblolly pines.

Basis	Trait	
	Percent infected	Galls/tree
	Loblolly pine	
Family	0.25	0.56
Individual tree	---	0.17
	Slash pine	
Family	0.68	0.50
Individual tree	---	0.36

Table 7.--Breeding values for fusiform-rust traits of six loblolly and six slash pines as calculated from fifth-year data from half-diallel crosses.

Parent number		Trait			
		Percent infected		Galls/tree	
		Loblolly	Slash	Loblolly	Slash
1	(R)[1/]	88.1	35.2	4.66	0.51
2	(S)	100.0	82.6	8.21	2.07
3	(R)	79.7	3.8	3.12	0.00
4	(R)	70.3	37.5	1.36	1.19
5	(S)	93.9	99.8	6.20	3.79
6	(S)	94.5	100.0	4.88	3.91
Mean:	R	79.4	25.5	3.05	0.57
	S	96.1	94.1	6.43	3.26

[1/] R = rated resistant and S = rated susceptible to fusiform rust.

The contrast between mean breeding values of the R and S parents was not nearly as great with loblolly pine as with slash pine for percentage infection. The loblolly pine R clones averaged 79 percent infection and the S clones 96 percent (Table 7). The contrast between R and S parents for number of galls per tree was similar for the two species, but progenies from the loblolly parents are expected to average about twice as many galls per tree as progenies from the slash pine parents.

DISCUSSION

The breeding values in Table 7 indicate that progenies from the R loblolly parents would be much more likely to get at least one fusiform rust infection per tree than would be progenies from the R slash parents. The number of parents screened in selection was not large in either case, so these results do not necessarily indicate that it is easier to find highly resistant slash pines than it is to find highly resistant loblolly pines. The heritability estimates (Table 6) are quite high for slash pine, promising large gains per generation from selecting and breeding for resistance to fusiform rust in these families. One might argue that these parents had an unusually wide spread in resistance, resulting in inflated heritability estimates. But when resistant slash pines were crossed with other resistant ones, they produced resistant offspring. These heritabilities, of course, apply only to this study.

Selection for rust-free individuals is expected to produce lower gains per generation in the loblolly than in the slash pine families. A family heritability of 0.56 for galls per tree, however, indicates that selection to reduce the number of galls per tree would produce large gains in loblolly pine. In another loblolly study involving a half-diallel cross among 10 parents, with the progenies planted in the same area as this study, the heritability of the family mean galls-per-tree trait was 0.56. Heritability of the rust-free percentage trait, however, was 0.62 in that study, much higher than the 0.25 in the present study (Sluder 1981).

The significant variation in specific combining abilities indicates that some nonadditive variation in rust resistance is present in both species. It may be that only a few loci control resistance to fusiform rust. Nonadditive variation is difficult to use in applied breeding programs. Perhaps the SCA variation would be useful in studies of such subjects as dominance, mechanisms of resistance, or number and location of resistance genes.

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Monoterpene Composition and Fusiform Rust Resistance in Slash Pine

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Abstract. Cortical monoterpene composition was determined for 43 relatively-resistant and 42 relatively-susceptible slash pine clones selected on the basis of their fusiform rust resistance breeding values. Fifty-seven percent of the clones having high proportions of β-phellandrene were relatively resistant, while only 15 percent of the low β-phellandrene clones were relatively resistant. Reasons for the relationship are not clear, but presence of the high β-phellandrene gene may be linked with other traits more directly involved with resistance. Certain combinations of monoterpenes, including particular minor constituents, may be more indicative of resistance than β-phellandrene alone, but further work is needed. For the present, tree improvement workers should consider determining monoterpene composition of candidate trees prior to progeny testing, and culling trees with low β-phellandrene content.

Additional Key Words: α-phellandrene, α-pinene, β-phellandrene, β-pinene, camphene, ***Cronartium quercuum*** f. sp. ***fusiforme,*** limonene, myrcene, ***Pinus elliottii*** var. ***elliottii, P. taeda,*** sabinene.

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VIRULENT ISOLATES OF CRONARTIUM QUERCUUM F. SP. FUSIFORME MAY IDENTIFY DIFFERENT RESISTANCE GENES

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Abstract.--Aeciospore isolates from six single fusiform rust galls varied in virulence towards 20 resistant loblolly pine families. Susceptibility of the pine families expressed as the percentage of seedlings with galls 6 months after inoculation, varied from immune (0% galls) to susceptible (77% galls). Some isolates were virulent on progeny of normally resistant pine selections, producing as many galls as on progeny of a susceptible selection. These isolates are relatively host specific and further host specificity should be possible by creating single spore lines.

The major benefit of identifying different resistance genes is to develop broadly based resistance in the pine population to reduce the possibility of catastrophic epidemics. An immediate benefit of identifying different resistance mechanisms is the gain we can expect in a breeding program that combines different resistance genes. Some controlled crosses between resistant trees have produced significantly more resistant progeny than do other crosses. The improvement appears to be due to combining resistance genes controlling different resistance mechanisms.

INTRODUCTION

In basic plant pathology, virulence is defined as a quantitative measure of pathogenicity, i.e., the ability to cause disease. In plant breeding, virulence is a quality of a pathogen to overcome defense mechanisms (resistance) in a host plant. H.H. Flor's (1956) pioneering research on flax rust demonstrated that a single virulent gene enabled a pathogen to infect an otherwise resistant host. Hence, the gene-for-gene hypothesis: for every gene for resistance in the host plant there is a corresponding and specific gene for virulence in the pathogen. Two characteristics of plant parasite systems with known gene-for-gene relations are: (1) Resistance is usually dominant and controlled by single genes. (2) Virulence is usually recessive and controlled by single genes (Christ et al 1987). This situation is demonstrated in the following table.

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Table 1. Resistant (R) or susceptible (S) responses of four varieties of a plant to four isolates of a pathogen with various combinations of virulence (V) and avirulence (v) genes towards the resistance genes in the varieties.

Pathogen	Plant			
	R_1R_1	R_1r_1	r_1r_1	R_2R_2
v_1v_1	S	S	S	R
V_1v_1	R	R	S	R
V_1V_1	R	R	S	R
v_2v_2	R	R	S	S

Fusiform rust and various cereal rust fungi are macrocyclic, heteroecious rusts. They have five different spore stages and their life cycle is completed on two different host species. On flax and cereals, the urediniospores occur on the economically important host species. Urediniospores are repeating spores since they can infect other plants of the same host species (wheat to wheat). The repetition enables virulent strains of the rust to spread rapidly within a susceptible host population. Urediniospores are dikaryons with an N+N nuclear condition. These nonfused nuclei function as though they were fused. Thus in Table 1 the pathogen could have v1v1, V1v1, or V1V1. Plants with the R1R1 or R1r1 genomes would be resistant to the latter two genotypes but susceptible to the former, since virulence is a recessive trait. Cereal pathologists and geneticists monitor the rust population and then utilize varieties of wheat that are resistant to strains of rust present in wheat growing areas.

Loblolly and slash pines are infected by basidiospores of Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme, the fusiform rust fungus. Spread from pine to pine does not occur. Basidiospores are products of meiosis and are haploid. If the gene-for-gene relationship is present in this host-pathogen system, then a basidiospore can be either only V or v towards resistance genes in the pine host. Avirulence towards resistance genes is usually more commonly encountered than is virulence. Only two of 56 single-gall isolates had virulence towards family 11-20 (Powers et al 1977). However, several authors (Powers et al 1977, Powers 1985, Snow et al 1970, 1975) have shown variations in virulence towards resistant pine families among isolates of fusiform rust. One of the reasons we more commonly find avirulence is that we often use mass inoculum composited from multiple galls. In the concentrated basidiospore spray (CBS) system we routinely use aeciospores collected from 8 to 30 galls. If virulence genes are present in only one of eight galls, the composite inoculum does not express the virulence (Matthews et al 1979, Powers et al 1977, Kuhlman in press). This failure to express virulence is analogous to expecting a mass pollination to show the superior pine growth from one of 8-30 pollen sources. In field progeny tests, trees are exposed to inoculum originating from many galls which could also mask the presence of virulence genes. However no field test nor any CBS test can make a regionwide evaluation of rust susceptibility of any set of pine families. There

simply is too much variation in the virulence of the pathogen population to make any such evaluation possible. What we can do is to select for rust resistance in field tests and CBS inoculation tests. Then virulent isolates can be used in CBS tests to identify selections with different genes or mechanisms for resistance.

The objective of the present study was to group rust resistant loblolly pine families on the basis of their variations in susceptibility to basidiospores from single-gall aeciospore isolates.

METHODS

Rust Isolates

Six single-gall aeciospore isolates with virulence towards resistant pine families were selected (Table 2). Virulence towards resistant loblolly (Pinus taeda L.) or slash pine (P.elliottii Engelm. var. elliottii) families had been demonstrated when the isolate caused a significant increase in the number of seedlings with galls over that caused by a control isolate.

Table 2. Response of resistant pine families to single-gall isolates as indicated by percentage of seedlings galled by the virulent isolate compared to those galled by isolates avirulent towards that resistance source (control).

Pine family	Virulent isolate	Virulent/Control Galled(%)	Ratio
Loblolly			
10-5	LHNC-2	83/42	1.98
11-20	SC-35	73/34	2.15
29R	0-1	87/39	2.23
2318	152-102	46/35	1.31
Slash			
3327-13	3327-13	57/33	1.73
10-226	10-226	85/45	1.89

Pine Families

Seeds from each of 21 loblolly pine families were stratified, germinated, and planted 20 seedlings per flat.

Experimental Design

21 Loblolly pine families
6 Rust isolates
126 Treatments

5 Replications/treatment
630 Flats

20 Seedlings/flat
12,600 Seedlings

Inoculation

Stored aeciospores of the six rust isolates were rehydrated prior to inoculating northern red oak (Quercus rubra L.) leaves. Basidiospores produced by these infections were concentrated at 50,000 per ml. for the CBS inoculation of the 6-week old pine seedlings. All 126 flats in a replication were inoculated on one day.

Data on symptom expression were taken 3 and 6 mo. after inoculation. A final reading will be made at 9 mo. The percentage of seedlings with galls after 6 mo. in each flat was transformed to an arc sin value prior to analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) comparisons.

RESULTS

Six months after inoculation, seedlings with galls within the 126 family x isolate treatments varied from 0-77% (Table 3). Susceptible family 4666-4 had the highest frequency of seedlings with galls when Dnoculated with each of the six isolates (Table 4).

Table 3. Ranges and means by isolates of percentages of seedlings with galls 6 mo. after 21 loblolly pine families were inoculated with each of six single-gall aeciospore isolates.

	Isolate					
Value	0-1	LHNC-2	SC-35	152-102	3327-13	10-226
Range	0-70	19-77	5-71	3-77	12-73	0-74
Mean	28	41	33	37	34	29

The ANOVA indicated all sources of variation (replication, isolate, family and isolate x family) were highly significant (P>0.0001). Therefore DMRT were used to compare isolate within family and family within isolate differences.

Table 4. Effects of family (F) and fungal isolate (I) on the percentages of seedlings with galls in 21 loblolly pine families 6 months after inoculation with basidiospores derived from six single-gall aeciospore isolates of Cronartium quercuum f. sp. fusiforme.

		Fungus isolate																	
	Resistance	0-1			LHNC-2			SC-35			152-102			3327-13			10-226		
Family	Source	%	F[a]	I[b]	%	F	I	%	F	I	%	F	I	%	F	I	%	F	I
42R	42R	26	B	b	69	A	ab	26	B	d-g	50	AB	bcd	35	B	b-g	31	B	b-f
11-41	11-41	32	AB	b	39	A	c-g	6	C	hi	3	C	h	26	AB	e-h	20	B	d-g
11-20	11-20	32	B	b	42	B	c-f	63	A	ab	32	B	c-g	36	B	b-g	29	B	b-f
4666-4	4666-4	70	A	a	77	A	a	71	A	a	77	A	a	73	A	a	74	A	a
152-60	TFS	18	A	b	20	A	g	15	A	ghi	19	A	fg	17	A	h	23	A	c-g
151-791	HH	17	A	b	19	A	g	29	A	d-g	25	A	efg	20	A	gh	32	A	b-f
10-6	10-6	24	A	b	32	A	efg	34	A	c-f	40	A	b-f	28	A	d-h	38	A	b-e
153-190	10R	19	AB	b	30	A	efg	33	A	c-g	28	A	efg	19	AB	gh	10	B	gh
153-517	7-56	26	BC	b	46	A	c-f	16	C	fgh	36	AB	c-g	28	ABC	e-h	13	C	fgh
10-5	10-5	30	C	b	60	A	abc	39	ABC	cde	51	AB	bc	52	AB	bc	35	BC	b-e
152-387	10-5	0	B	c	36	A	d-g	5	B	i	24	A	efg	31	A	c-h	0	B	i
153-424	29RX10-5	27	A	b	29	A	efg	19	A	efg	25	A	efg	26	A	e-h	7	B	h
29R	29R	55	A	a	39	A	c-g	54	A	bc	41	A	b-e	40	A	b-g	41	A	bc
151-576	T605	29	AB	b	41	A	c-f	36	AB	c-f	18	BC	g	12	C	h	19	BC	efg
151-307	1495-35	29	A	b	25	A	fg	26	A	d-g	28	A	d-g	23	A	fgh	41	A	bcd
151-334	T601	25	AB	b	37	AB	d-g	46	A	bcd	30	AB	c-g	19	B	gh	27	AB	b-f
151-627	2318	21	B	b	42	A	c-f	30	AB	d-g	43	A	b-e	38	A	b-g	23	AB	c-g
151-445	10-31	24	B	b	42	AB	c-f	26	AB	d-g	43	AB	b-e	46	A	b-f	35	AB	b-e
11-10	11-10	26	A	b	36	A	d-g	44	A	bcd	52	A	bc	51	A	bcd	49	A	b
1995-5	1995-5	30	D	b	51	ABC	b-e	37	BCD	c-f	59	A	ab	55	AB	b	32	CD	b-f
151-438	11-9	34	B	b	56	A	bcd	37	AB	c-f	53	AB	bc	47	AB	b-e	38	AB	b-e

[a]Within family treatments (horizontal comparisons) isolate percentages with the same letter (A-D) do not differ significantly according to Duncan's Multiple Range Test (P=0.05).

[b]Within isolate treatments (vertical comparisons) family percentages with the same letter (a-i) do not differ significantly according to Duncan's Multiple Range Test (P=0.05).

Some families had distinctly different responses to the six isolates. Family 42R (a selection by Harry Powers and John Kraus) was highly susceptible to isolates LHNC-2 and 152-102 but it was resistant to the other four isolates (Table 4). Note that family 42R's response to LHNC-2 was not different from susceptible family 4666-4's response to that isolate. Westvaco's family 11-41 was resistant to all isolates and highly resistant to isolates SC-35 and 152-102. Family 11-20 was as susceptible as 4666-4 to isolate SC-35 but it was resistant to the other five isolates. These were four distinct responses by the first four families. This is the type of identification of different resistance responses hoped for with virulent isolates.

Whereas family 4666-4 was uniformly susceptible to the six isolates, three families (152-60, 151-791 and 10-6) were uniformly quite resistant (Table 4). Families 153-190 and 153-517 were also quite resistant to all isolates but 153-517 was highly resistant to isolates 10-226 and SC-35, and 153-190 was highly resistant to 10-226.

Families 10-5 and 29R were most suceptible to isolates LHNC-2 and 0-1,respectively (Table 4). Family 10-5 was resistant to isolates 0-1, SC-35 and 10-226 whereas family 29R did not vary significantly in susceptibility to the six isolates. Tree 152-387 is an open-pollinated progeny of 10-5 that has produced highly resistant seedlings against our standard inoculum (3% with rust galls). In this study progeny of 152-387 were immune to isolates 0-1 and 10-226, highly resistant to SC-35, and resistant to the other three isolates. Tree 153-424 is a full sib of 29R and 10-5, its progeny have averaged less than 20% with galls with our standard inoculum. In this study family 153-424 was most resistant towards isolate 10-226, but was also very resistant towards all sources.

Both of these progenies of 10-5 in our seedling seed orchard have greater resistance to some of the six isolates than does 10-5. That is, the progeny of 152-387 were more resistant than those of 10-5 to all isolates except 3327-13. Similarly, progeny of 153-424 were more resistant than those of 10-5 to isolates LHNC-2, 152-102, 3327-13 and 10-226, and this family (153-424) was more resistant than 29R to 0-1, SC-35 and 10-226. In our routine screening with a composite isolate, we have tested 24 full-sib families of 29Rx10-5. Half of these, including 153-424, averaged galls on only 13% of the seedlings whereas the other 12 families averaged 41% with galls. These results suggest that half the families have resistance genes from both parents whereas the other half have resistance from only one parent.

Single-gall isolates were used in this study because they had shown enhanced virulence towards some resistant pine families. Single-gall isolates are probably quite heterogeneous, since a gall may have been initiated by more than one basidiospore and spermatization occurs annually to add new genomes. Studies are now underway to isolate single aeciospore lines of the pathogen each with a genome of two nuclei. Single-aeciospore lines will help us to determine if the gene-for-gene concept applies to the fusiform rust disease. Differences in virulence among these lines should be due to a few genes.

CONCLUSION

Six virulent isolates of C. q fusiforme indicated many different sources of resistance among 21 loblolly pine families. This approach to examining sources of resistance should help to insure that diverse sources of resistance are present in rust resistance orchards. The fungus has a tremendous potential for variation so many sources of resistance may be necessary to prevent epidemic outbreaks of rust in putatively resistant plantings.

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COMPARING FIRST-YEAR GROWTH OF BARE-ROOT AND CONTAINER PLANTINGS OF SHORTLEAF PINE HALF-SIB FAMILIES

J. C. Brissette and J. P. Barnett[1/]

Abstract.--Shortleaf pine seeds were collected from several ramets of clones identified as the female parents of seedlings that were either relatively good or relatively poor survivors in full-sib progeny tests. Seedlings from six such half-sib families were grown as both bare-root and container stock and planted on two sites in the Ouachita Mountains of Arkansas. When outplanted, the bare-root seedlings had greater mean height and root collar diameter than the container seedlings. However, the container seedlings had greater mean root volume and a more favorable shoot-to-root balance than the bare-root stock. After one growing season in the field, survival of all the families from both stock-types exceeded 94% at both locations. The container seedlings grew larger than the bare-root seedlings on both sites. Among the families, groundline diameters differed on one site, and family and stock type interacted in their effects on height on the other site.

Keywords: Pinus echinata Mill., family planting, stock-type comparison, Ouachita Mountains.

INTRODUCTION

On the Ouachita and Ozark National Forests in Arkansas and Oklahoma, shortleaf pine is usually planted on south- and west-facing slopes where soil moisture is often limited. One advantage cited for container versus bare-root southern pine seedlings is better performance on harsh, drought-prone, planting sites (Barnett and Brissette 1986). Superior survival and growth of container seedlings over bare-root stock on dry sites has been shown for longleaf pine (Amidon et al. 1982, Goodwin 1976, 1980), and for loblolly pine (Barnett 1980, Goodwin 1976, South and Barnett 1986).

Shortleaf pine has not been compared in as many stock-type trials as the other southern pines. Ruehle et al. (1981) compared container and bare-root shortleaf pine in an ectomycorrhizae study on two sites in the Ouachita Mountains. The container seedlings survived better and grew taller and to a larger diameter than the bare-root stock on the site with the best soil moisture characteristics. However, on the site with the drier moisture regime, the container seedlings were poorer than the bare-root seedlings in both survival and growth. The authors concluded that the container seedlings, which were considerably smaller, required more intensive site preparation to perform as well as the larger bare-root stock.

The use of half-sib family seed collections, rather than seed orchard mixes, for loblolly pine reforestation has been increasing for the past several years. One advantage of maintaining family identity is that variation in rates of seed germination and seedling growth is reduced. Thus, nursery culture can

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be tailored to individual families, or groups of families, to grow seedlings of higher quality than possible when a bulked seed collection is sown. In a previous study with a bulked seed orchard lot of shortleaf pine, our research seedlings were markedly more variable than the half-sib families of loblolly pine growing in nearby operational nursery beds. Therefore, we decided to make half-sib family collections for future shortleaf pine seedling research.

The primary objective of this study was to compare the field performance of shortleaf pine seedlings produced as bare-root and as container stock and planted on typical mountain reforestation sites in Arkansas. Additionally, we wanted to determine if family and stock-type interacted in their effects on survival and growth in the field.

METHODS

Family Selection

Seed collections were made at the USDA Forest Service Ouachita and Ozark seed orchard located near Mount Ida, Arkansas. The seed orchard is organized by geographic sources. Separate blocks in the orchard consist of selections from the Ozark National Forest and the east and west sides of the Ouachita National Forest. The geographic source of a family can be determined from the identification number; the 100's are east Ouachita, the 200's are west Ouachita, and the 300's are from the Ozark. Because no growth data were available when these selections were made, seed orchard clones were chosen on the basis of the survival of their progeny in full-sib tests planted on both forests. To be considered, a clone had to be the female parent in at least three tests. Progeny survival was compared to the average survival of each test. Thus, families that survived better than average in a test had relative survivals of greater than 100, and those that survived below average had relative survivals of less than 100. The overall average relative survival for progeny of the same female was the selection criterion.

In 1985, seeds were collected from several ramets of clones with an average relative survival greater than 100 and from others with an average less than 100. Seed processing was done at the Forest Service's Southern Forest Experiment Station laboratory in Pineville, Louisiana. Six half-sib families are in this study, two from each geographic source. Three are relatively good survivors (115, 219, and 322), and three relatively poor survivors (103, 202, and 342). The seeds were stratified for 35 days before sowing in the bare-root nursery.

Bare-root Seedling Production

The staff of the Weyerhaeuser Company Magnolia Forest Regeneration Center in southwest Arkansas grew the bare-root seedlings. The seeds were sown April 16, 1986, with a Weyerhaeuser-designed precision vacuum seeder. The machine sows eight drills with approximately 5 cm between the double row of seeds that make up each drill and about 15 cm between drills. The two outside drills on the 1.3-m wide nursery beds were planted with the seed orchard mix. The study families were assigned at random to the interior drills and re-randomized for each of seven blocks. Each family plot was 15.2 m long.

There was not enough time after the seeds were cleaned to conduct germination tests of stratified seed. Therefore, a germination rate of 90% was assumed, and the seeds were sown to achieve a density of about 250 seedlings per m^2. Nursery cultural practices, such as fertilization and root pruning, were applied based on the best judgment of the nursery manager. Top pruning was not done.

The seedlings were carefully hand lifted on February 2, 1987. They were packed in kraft-polyethylene (K-P) bags and kept in cold storage (about 3 °C) in Pineville until they were transported to the sites for planting.

Container Seedling Production

The container seedlings were grown at the Forest Service laboratory in Pineville. The containers were hand seeded April 23, 1986, into Ray Leach[2/] "Stubby" cells filled with a 1:1 peat-vermiculite medium. The volume of each cell is approximately 115 cm^3. One tray holds 98 of these cells at a density of about 500 per m^2. Each tray of cells was sown with a single family, with five replications of each family.

The containers were kept in a greenhouse for 2 weeks until germination was complete. They were then moved to raised benches outside in full sunlight where the seedlings were grown and hardened-off. Container southern pine seedlings grown under full sunlight have greater shoot and root dry weights than comparable seedlings grown in shaded greenhouses (Barnett 1989). Except for growing them outside, the seedlings received the culture regime recommended by Barnett and Brissette (1986).

Planting Sites

The plantings were on two typical National Forest sites in Arkansas. One site is on the Magazine Ranger District of the Ozark National Forest about 30 km southwest of Russellville. The other is on the Winona Ranger District of the Ouachita National Forest about 65 km southeast of Russellville and about 40 km west of Little Rock. Although most of the Ozark National Forest is in the Boston Mountains, the Magazine District is south of the Arkansas River and, therefore, in the Ouachita Mountains. Both sites are on gently rolling to rolling terrain with shallow, rocky soils. They were clearcut and site prepared as part of commercial operations. Both sites were ripped during site preparation.

On the Magazine site, the container seedlings were planted on December 10, 1986, and the bare-root stock on February 11, 1987. On the Winona site, the containers were planted on December 9, 1986, and the bare-root seedlings on February 11, 1987. The split planting was necessitated by the busy work schedule that winter. Consequently, stock-type is confounded with planting date in this study. For each family by stock-type combination, a 25-seedling row plot was planted.

[2/] The use of firm or corporation names is for the reader's information and convenience. Such use does not constitute official endorsement or approval by the U.S. Department of Agriculture of any product or service to the exclusion of others that may be suitable.

An electronic weather station is located at the Winona site within several meters of the study. It collects data on a 5 minute scan interval, then each hour records the means from several sensors, including several air and soil temperatures, relative humidity, soil matric potential, and wind speed and direction. Precipitation is recorded as it occurs.

Brissette and Carlson (1987) showed that the root growth potential (RGP), measured as the number of new roots greater than 1 cm long, of bare-root shortleaf pine seedlings averaged only 0.6 after 28 days at 10 °C. However, at 20 °C mean RGP for the same period was 11.7. At the Winona site, the daily mean soil temperature at a depth of 15 cm did not stay above 10 °C for more than two consecutive days until March 7. Thus, although the container seedlings were planted in December and the bare-root seedlings in February, new root elongation for both probably began at about the same time. Therefore we believe that confounding stock-type and planting date did not seriously affect the results of this study.

Measurements and Analyses

Seedling size at outplanting has a tremendous impact on first-year growth. Therefore, shoot length, root-collar diameter, and root volume were averaged for a 50-seedling sample of each family from each stock-type. We used the square of the root-collar diameter multiplied by the shoot length (D^2H) divided by root volume as an index of the shoot-to-root balance (S/R) of each seedling. After the first growing season, survival, total height, and groundline diameter of each planted tree were measured.

The experimental design for evaluating seedling morphology was completely random with each seedling an experimental unit. Stock-type, family, and the interaction between the factors were compared for each measured characteristic and for S/R with analysis of variance (ANOVA). The model had 588 degrees of freedom for error (dfe). Because of the selection method, family is considered a fixed effect in all the ANOVA models discussed.

The field data were analyzed separately for each site. The design at both locations is a split-plot in six randomized complete blocks. The experimental units are the 25-tree plots and ANOVAs for survival, total height, and groundline diameter were performed on the plot means. Stock-type is in the whole plot with 5 dfe. Family and the stock-type x family interaction are in the subplot with 50 dfe.

Survival data were transformed using the arc sine of the square root of the proportional value. This transformation ensures that the proportions are close to being normally distributed with homogeneous variance (Freese 1967). A linear contrast with the same mean square error (MSE) as the subplots was used to determine whether the field survival of families selected as relatively good survivors differed from those selected as relatively poor survivors. For F-tests of the family main effect with a $p \leq 0.05$, family means were separated using Duncan's multiple-range test.

RESULTS AND DISCUSSION

Seedling Morphology

The mean morphological characteristics are shown in Table 1. The stock-type x family interaction was significant for each of the measured characteristics and for the S/R. For shoot length, root collar diameter, and root volume the interaction explains 15%, 9%, and 8% of the total variation, respectively. For the S/R, the interaction accounts for less than 2% of the total variation. However, because of the large number of dfe, the F-test was very powerful. With an MSE of 0.316, the F value was 94.76, and p=0.0. Stock-type explains 12%, 17%, 19%, and 59% of the variation in shoot length, root-collar diameter, root volume, and S/R, respectively. Family accounts for 17%, 11%, 8%, and 4%, of the variation in those characteristics. The much more favorable shoot-to-root balance of the container seedlings probably explains why they often outperform bare-root seedlings on harsh sites.

Table 1. Morphological characteristics at the time of outplanting of container (Cont) and bare-root (Bare) shortleaf pine seedlings from selected half-sib families.

	Characteristic and stock-type											
	Shoot length			Diameter			Root volume			Shoot-to-root		
Family	Cont	Bare	Mean	Cont	Bare	Mean	Cont	Bare	Mean	Cont	Bare	Mean
	-------cm-------			------mm-----			-----cm^3-----			------ratio-----		
103	24.4	25.0	24.7	3.8	4.4	4.1	3.6	2.2	2.9	1.06	2.40	1.73
115	21.9	29.4	25.6	4.0	5.4	4.7	3.5	3.4	3.5	1.05	2.61	1.83
202	19.7	26.6	23.1	3.8	4.7	4.2	4.3	2.9	3.6	0.70	2.16	1.43
219	22.5	20.2	21.4	3.9	3.8	3.8	3.5	1.7	2.6	0.98	2.03	1.51
322	20.9	28.3	24.6	3.6	5.1	4.4	3.1	3.0	3.1	0.93	2.74	1.84
342	19.4	20.2	19.8	3.7	4.0	3.8	3.6	1.7	2.7	0.77	2.06	1.41
Mean	21.5	24.9	23.2	3.8	4.6	4.2	3.6	2.5	3.1	0.91	2.33	1.62

The size of the container seedlings in this study contrasted markedly with those planted by Ruehle et al. (1981), where container seedlings averaged only 12.3 cm tall and 2.0 mm in diameter. Their bare-root seedlings were similar to ours, averaging 23.4 cm tall and 5.1 mm in diameter.

Magazine Site

First-year survival on the Magazine site was excellent (Table 2). Such high survival indicates that the seedlings of both stock-types were of excellent quality and that the site had enough soil moisture during establishment. The interaction between stock-type and family was not significant (MSE=0.0135, F=0.93, p=0.47), and families did not differ (F=1.20, p=0.32). The linear contrast between families selected as relatively good and poor survivors showed no difference (F=0.05, p=0.82) between the two groups. The stock-types had similar survival (MSE=0.014, F=3.13, p=0.14). The ANOVA model explains only 29%

of the variation in survival. Mortality was so low that it cannot be attributed to either treatments or environmental factors.

First-year total heights averaged from 30 to 39 cm (Table 2). With an MSE of 6.658, there was no significant stock-type x family interaction (F=1.23, p=0.31), and families did not differ (F=1.73, p=0.15). However, the container seedlings were taller (MSE=9.240, F=90.01, p=0.0002). Compared to the average initial shoot lengths, the container seedlings grew an average of 17 cm for an 81% increase in height. The bare-root seedlings grew an average of 7 cm, or increased 29%. The ANOVA model explains 76% of the variation in first-year height, with stock-type alone accounting for 61%.

Table 2. First-year survival, total height, and groundline diameter of half-sib families of shortleaf pine grown as container (Cont) and bare-root (Bare) stock and planted on the Magazine Ranger District of the Ozark National Forest.

	Characteristic and stock-type								
	Survival			Height			Diameter		
Family	Cont	Bare	Mean	Cont	Bare	Mean	Cont	Bare	Mean
	----------%---------			------cm------			--------mm-------		
103	97.6	95.3	96.0a[1/]	39	30	34a	6.0	4.7	5.4bc
115	98.7	96.7	97.7a	38	33	36a	6.1	5.2	5.7ab
202	98.7	96.7	97.7a	38	32	35a	5.7	5.0	5.3bc
219	99.3	96.0	97.6a	37	30	33a	5.7	4.7	5.2c
322	97.3	98.7	98.0a	38	33	35a	6.2	5.5	5.8a
342	99.3	98.0	98.6a	38	30	34a	6.3	4.5	5.4bc
Mean	98.3p[2/]	96.9p	97.6	38p	31q	35	6.0p	4.9q	5.5

[1/] Family means for a given characteristic followed by the same letter are not significantly different at p=0.05.
[2/] Stock-type means for a given characteristic followed by the same letter are not significantly different at p=0.05.

Groundline diameters averaged from 4.5 to 6.3 mm after the first growing season (Table 2). The stock-type x family interaction was not significant (MSE=0.2486, F=1.90, p=0.11). Families differed (F=3.01, p=0.0188); Family 322 was the largest and Family 219 the smallest (Table 2). However, compared to average root-collar diameter at outplanting, both families increased an average of 34%. The container seedlings had a larger average diameter than the bare-root stock (MSE=0.558, F=20.91, p=0.0017). The mean diameter of the container seedlings increased 58% compared to the mean root-collar diameter at outplanting, while the bare-root seedlings increased 11%. Stock-type explains 47%, and family 8% of the total variation in first-year groundline diameter.

Winona Site

Survival was also excellent at the Winona site (Table 3). There was no stock-type x family interaction (MSE=0.018, F=0.17, p=0.97), or difference due to family (F=0.28, p=0.92). There was also no difference between the relatively good and relatively poor surviving families (F=0.00, p=0.95). The container

seedlings survived better than the bare-root stock (MSE=0.010, F=21.90, p=0.0054). Although the difference is statistically significant, it is of little practical importance. The ANOVA model explains 31% of the variation.

There was a significant stock-type x family interaction in total height (MSE=8.162, F=2.42, p=0.0483). Although each family was taller from containers, the ranking of the families differed. The biggest differences were in Families 219 and 322. Family 219 was tallest from containers, but from bare-root it was the second shortest. Family 322 was shortest from containers, but the second tallest bare-root family. The interaction explains only 3% of the total variation in total height. Although such an interaction is not important to silviculturists, it may be of interest to geneticists, especially if early evaluations of families are planned. Stock-type accounts for 66% and family 4% of the variation in total height. First-year mean total heights represent growth of 35% to 91% compared to average shoot lengths at outplanting.

Table 3. First-year survival, total height, and groundline diameter of half-sib families of shortleaf pine grown as container (Cont) and bare-root (Bare) stock and planted on the Winona Ranger District of the Ouachita National Forest.

	Characteristic and stock-type								
	Survival			Height			Diameter		
Family	Cont	Bare	Mean	Cont	Bare	Mean	Cont	Bare	Mean
	----------%---------			------cm------			--------mm-------		
103	98.0	95.3	96.6a[1/]	41	30	36	6.6	5.1	5.9a
115	99.3	96.0	97.6a	40	34	37	6.8	5.6	6.2a
202	100.0	95.3	97.6a	41	30	35	6.9	5.6	6.3a
219	98.7	96.0	96.4a	42	29	35	6.7	4.8	5.8a
322	98.7	94.0	96.4a	38	30	34	7.1	5.3	6.2a
342	99.3	96.0	97.6a	39	27	33	7.1	5.5	6.3a
Mean	99.1p[2/]	95.4q	97.3	40	30	35	6.9p	5.3q	6.1

[1/] Family means for a given characteristic followed by the same letter are not significantly different at p=0.05; no letter indicates a stock-type x family interaction at p=0.05.

[2/] Stock-type means for a given characteristic followed by the same letter are not significantly different at p=0.05; no letter indicates a stock-type x family interaction at p=0.05.

Mean groundline diameters ranged between 4.8 mm and 7.1 mm (Table 3). They were not affected by the stock-type x family interaction (MSE=0.383, F=0.57, p=0.72), or by family (F=1.51, p=0.20). The container seedlings had a larger mean diameter than the bare-root seedlings (MSE=0.746, F=57.56, p=0.0006). First-year diameter growth of the container stock represented an average 82% increase compared to the mean root-collar diameters at outplanting. The bare-root seedlings grew an average of 20%. The model explains 79% of the total variation in first-year diameter with stock-type accounting for 48%.

CONCLUSIONS

If quality seedlings are planted, either bare-root or container, shortleaf pine will survive well on prepared sites in the Ouachita Mountains. However, in this study container seedlings grew better than bare-root stock during their first growing season. The superior field performance of container stock is probably due to larger root systems and better balanced seedlings.

Differences in morphology suggest that half-sib families of shortleaf pine are different enough that maintaining family integrity during seedling production will improve the overall quality of the planting stock. Stock-type and family may interact to affect growth after outplanting on some sites. Although container shortleaf pine can be expected to outperform bare-root stock, the difference will be greater for some families than for others.

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GENERAL SESSION:
BIOTECHNOLOGY

ASSESSMENT OF THE STABILITY, FATE AND SURVIVAL OF A ROOT COLONIZING LACZY ENGINEERED PSEUDOMONAS AUREOFACIENS

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Abstract.--A genetic modification of a natural root-colonizing Pseudomonas aureofaciens bacterium was accomplished by inserting into the chromosome two genes from E. coli, lacZ and lacY. The engineered pseudomonad (Ps. 3732RNL11) is now able to utilize lactose as a sole carbon source. This has provided a powerful genetic marker that not only distinguishes the engineered strain from other fluorescent pseudomonads in the environment, but enable an investigator to effectively assess its fate, survival and genetic stability in the field. The natural resistance of the native strain to two antibiotics rifampicin and nalidixic acid, enhances detection, and provides a comparison of the lacZY monitoring system with more traditional recovery based on antibiotic resistance alone. On Nov. 2, 1987, an EPA-approved small-scale field study (< 1.5 acres) was initiated using this engineered strain. During the first planting of winter wheat, approximately 10 CFU OF Ps.3732RNL11 were introduced with the wheat seeds. Root colonization after the first week was about 10 CFU/g root. After three weeks, this level gradually declined to about 10 CFU/g root at harvest, 32 weeks after planting. A second crop rotation of soybean with no additional inoculum was then planted on the field site. Ps.3732RNL11 was found at a low level on the soybean roots during for the first three weeks, but was not detected through the remainder of the soybean growing season (20 weeks). After soybean harvesting, the soil was tilled and a third crop rotation, winter wheat, was planted without additional bacterial inoculum. Analysis of possible re-colonization of the wheat roots by residual Ps. 3732RNL11 in the field soil is now being examined. Movement of Ps.3732RNL11 from the point of initial inoculation was found to be very minimal, detected in rows immediately adjacent to the row inoculated in only 3% of more that 6,500 samples taken. No transfer of the engineered genes in the field to other indigenous microbes has been detected. The presence of the strain has not been observed in the field site plant free area, in water run-off of the field, irrigation pond water, or in/on upper plant parts.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM MAPPING OF LOBLOLLY PINE: METHODS, APPLICATIONS, AND LIMITATIONS

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Abstract.--A saturated restriction fragment length polymorphism (RFLP) linkage map for loblolly pine (*Pinus taeda* L.) is being constructed. RFLPs are mapped by classical segregation analysis in 3-generation pedigrees provided by the North Carolina State University- Industry Cooperative Tree Improvement Program. The map will provide a detailed description of the organization of the loblolly pine genome and serve as an important tool for tree breeding and gene transfer. It may be possible to use the map to identify quantitative trait loci (QTLs) and for marker-assisted early selection, although, several factors presently limit the utility of these applications in loblolly pine and other conifers.

Keywords: RFLPs, genetic mapping, *Pinus taeda* L.

INTRODUCTION

We are constructing a high-density genetic map for loblolly pine (*Pinus taeda* L.) based on restriction fragment length polymorphism (RFLP) markers. Loblolly pine was chosen among conifer species because of its commercial importance, immediate availability of 3-generation pedigrees, small genome size relative to many other conifers, and membership in the genus *Pinus*. Furthermore, loblolly pine is a likely candidate for improvement via gene transfer technology, which will be greatly facilitated by a genetic map.

Our goal is to understand more about the organization of conifer genomes. Aside from information obtained from karyotype analysis (Saylor 1972) and isozyme analysis (Adams and Joly 1980a, 1980b), little is known about the organization of the loblolly pine genome. By mapping a large number (200-300) of RFLP markers, we hope to obtain a detailed description of how the genome of this species is organized.

Aside from addressing basic questions on genome organization in loblolly pine, there will be direct applications of the genetic map. These include (1) the acquisition of a large number of new genetic markers for population genetic studies or for monitoring tree breeding activities, (2) identification of insertion sites following DNA transfer; and (3) use in

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conjunction with other technologies to isolate individual genes and chromosomal segments encoding quantitative trait loci (QTL). There may also be an opportunity to use RFLPs for marker-assisted early selection.

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

A restriction fragment length polymorphism (RFLP) is most easily envisioned as a simply inherited Mendelian trait viewed at the molecular level. RFLPs are variations in DNA sequence between homologous chromosomes and can be detected using standard molecular biology protocols. First, DNA samples are isolated from individual trees; needle tissue is commonly used for conifers, although DNA can be isolated from most tissues. Then, DNAs are cut into discrete fragments by adding a restriction endonuclease. These enzymes recognize specific DNA sequences (usually 4-8 base pairs) and cleave DNA at or near these sites. Over 500 restriction enzymes have been isolated, mostly from microorganisms. The DNA fragments are size fractionated by gel electrophoresis. Restriction fragments are then transferred to a solid support membrane by a technique called Southern blotting (Southern 1975).

The next step in detecting RFLPs is to hybridize Southern blots with DNA probes. Radioactively labelled DNA probes are hybridized to homologous DNA sequences on the blots. The unbound probe is washed from the blot before exposing it to X-ray film. The resulting autoradiogram reveals only DNA sequences that are homologous to the probe. Variations in the position of bands among DNAs on the autoradiogram are the result of DNA sequence variation and are thus called restriction fragment length polymorphisms. RFLPs are usually the result of a gain or loss of restriction sites due to mutations within recognition sites, or are due to small insertions or deletions.

RFLPs have numerous advantages over other types of markers for genetic mapping. Contrasted with isozymes and morphological markers, RFLPs provide a virtually unlimited number of genetic markers. RFLP markers are developmentally stable and display normal Mendelian inheritance. RFLPs generally exhibit multiple alleles, codominance, minimal pleiotrophic effects, and little if any environmental effect (Beckman and Soller 1983, 1986). At present, RFLPs are the most effective, if not the only, method available for constructing a saturated genetic linkage map for loblolly pine.

Several problems associated with RFLP mapping will, at first, limit the widespread application of this technology to forest trees. One is the need for experience in molecular biology methodology which is just being developed in forestry research organizations. These methods must then be adapted to forest trees. Finally, the cost of this technology can be prohibitively high for some research organizations.

METHODS FOR CONSTRUCTING AN RFLP MAP FOR LOBLOLLY PINE

Isolation of Conifer DNA

Total loblolly pine genomic DNA is prepared from fresh needle tissue following modifications in the procedure of Murray and Thompson (1980). Fresh needles are frozen under liquid nitrogen and ground to a fine-textured powder which is then dispersed in a sorbitol-based extraction medium. Organelles are collected by sedimentation centrifugation, resuspended, and lysed with sarkosyl to release the DNA. The DNA is purified by chloroform/octanol extraction and banding on CsCl-ethidium bromide isopycnic gradients before ethanol precipitation. Yield is typically 0.5 to 1.0 mg per gram fresh weight of tissue.

Southern Blots

Genomic DNA is digested with each of several restriction endonucleases that were selected on the basis of cost, their capacity to digest genomic DNA completely, and their ability to reveal RFLPs in an initial screening study. The enzymes chosen included EcoRI, EcoRV, HindIII, and BamHI. Total DNA is digested to completion according to the supplier's recommendations. Restriction fragments are separated by horizontal agarose gel electrophoresis and transferred to Hybond-N (Amersham) nylon membranes for hybridization analyses.

DNA Probes

A complementary DNA (cDNA) library was constructed from messenger RNA (mRNA) isolated from two-week-old loblolly pine seedlings. A cDNA library was chosen as the source of probes because of its enrichment for single-copy sequences, as opposed to a genomic library, which is ordinarily high in repetitive sequences. Random cDNA clones have also been shown to reveal as much or more variability than genomic clones (Landry et al. 1987). Double-stranded cDNAs were inserted into a plasmid vector for cloning. Amplified clones are prepared for hybridization by radiolabeling to high specific activity with ^{32}P-dCTP (3000 Ci/mmol) using the random primer technique (Feinberg and Vogelstein 1983).

Southern Blot Hybridization Analysis

Southern blots are hybridized according to established protocols (Maniatis et al. 1982). Blots are prehybridized at 42°C for 3-5 hrs. with a solution containing 50% formamide. The prehybridization solution is replaced with fresh solution containing the radiolabeled probe at a concentration of approximately 5 x 10^6 dpm/ml. Hybridization is continued at 42°C for 16-24 hrs. Aqueous hybridizations at 65°C can also be used. Blots are washed at high stringency and autoradiographed at -80°C for 1-6 days.

Loblolly Pine Pedigrees

The RFLP map will be based on a single 3-generation pedigree from the North Carolina State University Industry Cooperative Tree Improvement Program. Measurement of linkage requires the joint segregation of two or more heterozygous loci in at least one parent of a cross (Bailey 1961; Mather 1951). Therefore, the more heterozygous loci an individual parent tree has, the more information on linkage it contains. A rapid estimate of individual tree heterozygosity can be obtained from isozyme analysis. Thus, to maximize heterozygosity in the cross selected for RFLP mapping, 155 candidate parent-pairs (second generation selections) were assayed for isozyme heterozygosity at 39 loci. Parent-pairs had from 0 to 14 heterozygous loci; the five most heterozygous pairs were selected for additional screening for RFLPs. In this preliminary screening for selection of a pedigree, it was assumed that isozyme and RFLP variability are correlated. This assumption is supported by comparative estimates obtained for maize and tomato (Helentjaris et al. 1985).

The five selected parent-pairs were screened for RFLPs by using combinations of four enzymes (EcoRI, EcoRV, BamHI, and HindIII) and 40 probes. Fifteen combinations revealed a polymorphism between parent-pairs for at least one cross. The most variable parent-pair possessed 10 of the 15 RFLPs found and has been selected as the parent-pair for the mapping project. This cross also had the most heterozygous loci based on the isozyme data.

We have begun screening the selected cross for RFLPs with the collection of random cDNA probes. The probe x enzyme combinations that identify RFLPs will be assayed on blots containing DNA for 100 or more progeny to test for Mendelian segregation of RFLPs. The four grandparents (first-generation selections) of this pedigree will also be screened to provide information on phase of alleles in the parent trees. Parental screening and progeny analysis will require approximately three years to complete.

Data Analysis

RFLP segregation data will be subjected to genetic linkage analysis similar to that used for human pedigrees. Human and conifer pedigrees are both outbred, unlike many crops, and the phase of alleles is often unknown. A general approach to linkage computation, the method of maximum likelihood, was advanced by Haldane and Smith (1947). Elston and Stewart (1971) formulated a general algorithm for calculating likelihood of a given map. Several computer program packages have been developed to implement this algorithm, including LIPED (Ott 1976) and LINKAGE (Lathrop and Lalouel 1984). The Elston-Stewart algorithm, however, is not well suited to a multilocus analysis of a large number of loci because the computation time needed to calculate likelihoods increases exponentially with the number of loci. Lander and Green (1987) described an alternative algorithm and accompanying computer package, MAPMAKER (Lander et al. 1987; Lincoln and Lander 1987), for multipoint analysis which overcomes the limitations of the Elston-Stewart technique. Hulbert et al. (1988) used MAPMAKER to analyze RFLP loci in

lettuce downy mildew (Bremia lactucae), an outcrossing fungus. Data from the present study will be analyzed with the MAPMAKER package using a microcomputer.

Number of Markers Required for Genome Saturation

The number of RFLPs mapped depends upon the degree of saturation desired and the size of the genome. It has been suggested that if the map is used to identify QTLs, then the maximum distance between markers should be 0.2 to 0.4 Morgans (c = 0.1 to 0.2)(Soller et al. 1976; Soller and Plotkin-Hazen 1977). Lange and Boehnke (1982) developed the expression $n = \log(1-p)/\log(1-2c/k)$ to estimate number of markers where: n = number of markers required, p = proportion of a circular genome to be mapped, c = desired distance in Morgans between marker and QTL, and k = total length of genome in Morgans. Lange and Boehnke cautioned, however, that chromosome end effects increase this estimate by 20 to 30%.

The number of map units in the genome of loblolly pine (N = 12) has not been accurately determined. From isozyme linkage analyses, however, we estimate that the genome is approximately 2000 cM. Using the expression of Lange and Boehnke and assuming p = 0.9, c = 0.1, and k = 20, the corrected estimate is 275 markers. Since this estimate of genome size is based on limited data, it will be re-estimated once 50 to 100 RFLP markers have been mapped.

APPLICATIONS AND LIMITATIONS OF RFLPS IN TREE IMPROVEMENT

Genetic Markers

An important early application of the genetic map will be the acquisition of a large number of new genetic markers. To date, only a few morphological (Franklin 1970) and isozyme (Adams and Joly 1980a, 1980b) markers have been identified in loblolly pine. RFLP markers could improve the efficiency of clonal identification, parental verification in crosses, seed lot identification, and seed orchard studies (Adams 1981, 1983). More genetic markers will also improve estimation of other genetic parameters, such as recombination rates, genetic diversity, mutation rates, and segregation distortion. RFLP technology is currently expensive and labor intensive; thus isozymes will be the preferred marker where these constraints are present.

Construction of the initial RFLP map for loblolly pine will be very time consuming. However, once a full set (200-300) of RFLP probes has been identified and mapped, they will be potentially very useful as markers in other loblolly pine crosses and possibly in related pines. Mapping other crosses and related species should proceed much more rapidly than the initial map.

Identification of Quantitative Trait Loci

One of the most important, but longer term, applications of this map will be the opportunity to evaluate the potential for detecting linkages between RFLPs and quantitative trait loci (QTLs). Several QTL have recently been mapped in tomato by using either a regression approach (Nienhuis et al. 1987; Osborn et al. 1987; Tanksley and Hewitt 1988; Martin et al. 1989) or a new maximum likelihood method (Paterson et al. 1988; Lander and Botstein 1989). These analyses were conducted in either backcrosses or F_2's.

A theory has not been developed to detect linkages between RFLPs and QTLs in outbred populations in linkage equilibrium such as loblolly pine and other conifers. It may be possible to detect linkages between RFLPs and QTLs in specific crosses in which matings are made between trees of phenotypic extremes for the quantitative trait (Lander and Botstein 1989) and if the heritability of the trait is high and under the control of a small number of genes (Lander and Botstein 1986). An empirical test of the power of RFLP-QTL analysis in conifers should be made once the genetic map is completed. Specific traits and crosses should be chosen to maximize the opportunity of successfully detecting linkages. If this test proves successful, additional experiments could be designed to test for linkages between RFLPs and commercially important traits.

Marker-Assisted Selection

Forest tree breeders have continually searched for ways to select the best genotypes in breeding populations prior to rotation age. In loblolly pine, the approach has been to determine if correlations exist between the value of a trait expressed in a juvenile plant and a mature plant. If a strong juvenile-mature correlation can be established, then it may be possible to select for the trait in very young plants. Juvenile-mature correlations have been shown for traits measured in young plants (4-6 years) (Lambeth 1983; Lambeth et al. 1983; Foster 1986) and also for very young seedlings (<2 years) (Waxler and van Buijtenen 1981; Williams 1987,1988). First-year selection is currently practiced for fusiform rust resistance, but as yet, reliable first-year selection criteria have not been developed for yield or other commercial traits.

A second approach to early selection is marker-assisted indirect selection. This method has not been tried in loblolly pine because of the absence of genetic markers. This could change rapidly with the development of a saturated RFLP linkage map. The first step is to establish linkages between RFLPs and QTLs. Linkages between RFLPs and QTLs for commercially important traits have recently been demonstrated in tomato (Paterson et al. 1988). As noted earlier, however, linkages have been shown in F_2 and backcross populations derived from highly inbred lines. In this situation, the "high" QTL allele is expected to be in cis with one RFLP allele and the "low" QTL allele with the alternative RFLP allele. In conifer populations, RFLP and QTL alleles will essentially be in linkage equilibrium. Several designs have been suggested for outbreeding populations to overcome this problem (Soller and Genizi 1978; Soller and Beckman 1983; Beckman and Soller

1988); however, each has severe limitations that make them impractical to apply to conifers. Currently, we do not consider marker-assisted selection applicable to existing loblolly pine breeding programs. Only if breeding programs were limited to a very small number of elite pedigrees would it become feasible to develop maps for each pedigree and practice indirect selection on RFLPs linked to QTLs for traits of interest.

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DNA SEQUENCE DIVERSITY IN ALCOHOL DEHYDROGENASE GENES FROM PINES

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Abstract.--Molecular clones that represent ADH coding sequences (cDNA clones) from Monterey pine have been been isolated and characterized. These clones have been subsequently used to identify DNA fragments containing homologous sequences in samples of DNA prepared from needles of mature loblolly and Monterey pine trees. Relative to analogous studies of ADH from angiosperms, the number of fragments in pines is large. Just how these homologous sequences are related to each other is currently under investigation. Genomic libraries have been constructed in a bacteriophage vector (EMBL3) for both loblolly and Monterey pines. Several million recombinant phage were screened using our ADH cDNA clones as molecular probes. We identified a large number of clones containing sequences homologous to our probes, a result that is consistent with our studies of restriction fragments. Preliminary analyses of DNA purified from these clones suggest that many contain DNA sequences from different portions of the genome. In combination, these data suggest that ADH in pines may belong to a gene family that contains more members than suggested from studies of isozymes (i.e. 2-4 loci). Alternatively, pine ADH genes may themselves be very large, containing many or large intervening (non-coding) sequences in addition to a normal complement of coding sequences.

Keywords: Pinus taeda, Pinus radiata, ADH, molecular cloning, multi-gene family, genetic engineering, gene structure, genomic library.

INTRODUCTION

A basic understanding of gene structure and expression is a prerequisite for genetic engineering. Optimum utility from engineered novel (or foreign) genes that have been transferred into plants will come only if their expression can be regulated in a desired fashion. Likewise,

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endogenous genes identified as having important roles in plant growth can be subjected to genetic engineering only if their structure and expression are understood. The structure and regulation of a number of genes from angiosperms are being studied, including genes encoding alcohol dehydrogenase (ADH). We are studying the structure and expression of alcohol dehydrogenase (ADH) genes in loblolly (Pinus taeda) and Monterey (P. radiata) pines because information from angiosperms will help us to more readily understand similar properties of conifer genes.

ADH is the enzyme that catalyzes the reversible conversion of ethanol to acetaldehyde. When roots of many plants are deprived of oxygen, as would occur following flooding, ADH activity increases rapidly and dramatically (Bennett and Freeling 1988). The function of ADH under these circumstances is not clear, but may include the generation of cellular energy or the maintenance in intracellular pH (Bennett and Freeling 1988). In woody plants, ADH activity may be constitutive in stems and in leaves, perhaps because of anaerobic conditions in the vascular cambium (Kimmerer and Stringer 1988).

Much of the increased ADH activity following flooding is caused by increased transcription of ADH genes (see reviews by Freeling and Bennett 1985, Harry et al. 1988). Specific DNA sequences involved in the activation of these genes are being identified, including sequences in the promoter region (Walker et al. 1987) and in introns (Callis et al. 1987). Such studies have included transferring DNA sequences of ADH isolated from one species into evolutionarily similar and dissimilar species. For example, when regulatory sequences of ADH from corn (a monocot) were transferred into tobacco (a dicot), they failed to function properly, even though regulatory sequences of ADH from pea (another dicot) functioned properly in tobacco under identical circumstances (Howard et al. 1987, Walker et al. 1987). This has important implications for applying genetic engineering to conifers because genes that are engineered for optimal expression in angiosperms may not function properly, if at all, in gymnosperms.

ADH activity is induced by anaerobic stress in pines as it is in other plants, with the activity of one ADH gene being greatly increased relative to a second ADH gene (Harry et al. 1988, Harry et al., in preparation). As an initial step towards the characterization of pine ADH genes, we used an ADH cDNA from maize (pZML793, Dennis et al. 1984) to identify cDNA clones from Monterey pine (Harry et al. 1988, Kinlaw et al. 1988, and Kinlaw et al., in preparation). None of the identified clones contain the entire coding region from ADH. Three of the longer clones, designated RCS1025, RCS1019, and RCS1029 (hereafter abbreviated simple as 1025, 1019, and 1029) have been fully characterized (Kinlaw et al. in preparation). Based on its alignment with the maize sequence, the 531 bp insert of 1025 spans 518 bp of the protein coding region, including the ATG translational start site. The approximately 400 bp insert of 1019 overlaps with about 250 bp at the 3' end of 1025, continuing further downstream for another 150 bp. 1029 and 1019 are nearly identical. Comparisons of DNA sequences among pine, monocot, and dicot ADHs shows that 1025 and 1019-1029 probably represent two genes whose ancestral form diverged from the lineage leading to angiosperms shortly before monocots and dicots diverged (Kinlaw et al. in preparation). When

these pine cDNA clones were used as probes against Southern blots of genomic DNA from Monterey and loblolly pines, many homologous genomic sequences were identified (Harry et al. in preparation). This result was surprising since ADH belongs to a small gene family in the angiosperms studied to-date.

As part of our continued efforts to characterize ADH in pines, we have screened genomic libraries from Monterey pine and from loblolly pine. Consistent with our analyses of genomic Southern blots, we identified many genomic clones that share DNA sequence homology with our pine ADH cDNAs. We present here preliminary analyses of some of the many clones we have identified.

MATERIALS AND METHODS

Total cellular DNA was isolated from Monterey and loblolly pines using an extraction procedure described by Loopstra et al. (in preparation). For Monterey pine, we used cells from the suspension culture line we had used earlier as a source of our pine cDNA clones (Kinlaw et al. 1988, and Kinlaw et al., in preparation). This cell line has been maintained in culture for several years and was a gift from Dr. Robert Teasdale, Bond University, Australia. For loblolly pine, needles were collected from clone 7-56 in the NC State Cooperative Tree Improvement Program. For both species, the majority of the purified DNA was over 100 kilobases (kb) in length, as judged by gel electrophoresis (unpublished).

Libraries for both species were prepared using standard methods in the bacteriophage cloning vector EMBL3 (see Kaiser and Murray 1985 for a description of the methods). The Monterey pine genomic library was prepared under contract by Clontech, Palo Alto, California, while the loblolly pine was prepared by one of us (CAL). Briefly, DNA was subjected to partial digestion by restriction endonucleases (Sau3A or MboI), size fractionated by centrifugation, and ligated to BamHI digested "arms" of the bacteriophage cloning vector EMBL3. Recombinant phage were packaged in vitro before being mixed with E. coli host cells (strains K802 or K803) for plating. The Monterey pine library contains approximately 2.7 million independent recombinants while the loblolly pine library is several-fold larger.

The libraries were plated at an average density of approximately 50,000 recombinant phage (or plaque forming units, pfu) per 150mm petri dish. Two to four filter lifts (Hybond-N, Amersham) were made from each of 20-30 plates, but in all cases, duplicate filters were used in each subsequent DNA hybridization reaction. The filters were probed with [32]-P labeled DNA from purified inserts of the cDNA clones 1025 or 1029. A typical hybridization reaction consisted of 30 filters in a jar with 350 ml hybridization solution (6X SSPE or SSC, 5X Denhardt's, 200 ug/ml carrier DNA [salmon sperm, Sigma Chemical], and 0.5% w/v SDS, 65^{o} C, Maniatis et al. 1982) with labeled DNA from 300 ng of template. For large hybridization reactions such as these, we have found that placing nylon mesh (e.g. nylon window screens cut into circles) between the filters helps to eliminate background. After hybridization (18-24 hours), filters were washed in at least four changes of 0.5X SSPE (or SSC) with 0.1% SDS, 65^{o} C.

Following autoradiography, plaques showing positive hybridization signals on both replicate filters were identified and subjected to a second round of plating and screening. Secondary screens were done using a lower plating density (1,000 to 5,000 pfu per 150mm plate), and plaques that again showed positive hybridization signals to ADH cDNA probes were subsequently purified. Once a homogeneous phage stock was obtained, phage DNA was prepared from liquid lysates using a PEG "miniprep" procedure modified from Ausubel et al. (1989). Purified phage DNA was digested with various restriction enzymes (BRL, using the manufacturer's recommendations), subjected to electrophoresis (0.7-1.2% agarose in 1X TBE buffer containing 0.5 mg/l ethidium bromide, Maniatis et al. 1982). Fragments were visualized and photographed on a UV transilluminator (302 nm). Fragment lengths were estimated visually by comparing their migration distances to DNA standards included in the gel. Subsequently, DNA was blotted to to membrane filters (Hybond-N), probed with labeled ADH cDNAs, and the hybridizing DNA fragments were determined by autoradiography. Hybridizations and washes were done using high stringency conditions when pine cDNAs were used as probes, but the the stringency of the washes was reduced (4X SSC, 0.1% SDS, 65^{o}) when the maize clone pZML793 was used as a probe. Blots were probed first with either 1025 or 1029, then stripped (following the manufacturer's recommendations) and probed a second or third time. When pZML793 was used as a probe, it was always used last.

RESULTS AND DISCUSSION

We identified a large number of clones containing sequences homologous to our cDNA clones (Table 1). We considered as positive only those plaques with clear hybridization signals on replicate filters. Using this strategy, our frequency of false positives is very low (2-5% or less). The frequency of positive clones (number of positives per pfu screened) we identified from each library is remarkably consistent, as is the fraction of clones that show hybridization to both probes. Because 1025 extends further towards the 5' end of the gene than does 1029, we speculate that some of the additional DNA sequences identified by 1025 share homology with this region. We are not certain why both 1025 and 1029 hybridize to some genomic clones under the high stringency conditions we used in these experiments. The pine cDNAs share about 85% DNA sequence homology and clearly show different patterns of hybridization on identical Southern blots of genomic DNA. Nevertheless, results from hybridization experiments using plaque lifts (or blots of phage DNA following electrophoresis) and genomic DNA are not comparable.

Purified DNA prepared from homogeneous phage stocks has now been prepared from genomic clones of both Monterey and loblolly pines. As an initial step to characterize these clones, we have digested phage DNA with selected restriction endonucleases. SalI separates insert DNA (genomic DNA from pine) from that of the phage vector. Cloned pine DNA is visualized as one or more fragments, depending on the number of SalI cleavage sites contained in the insert. In addition, EcoRI or BamHI were used in combination with SalI because phage vector contains no recognition sites for either of these enzymes. In this way, vector and insert DNA are easily

distinguished, and fragment lengths of the insert DNA can be conveniently determined. It is important to distinguish DNA fragments formed by cleavage at the SalI site of the vector adjacent to the insert DNA from fragments formed by cleavage at internal recognition sites. Such fragments have been truncated in the cloning process and will be shorter than homologous sequences in vivo or that are represented as internal fragments in independent genomic clones.

Table 1. Number of positive plaques identified while screening genomic libraries of Monterey pine and loblolly pine using probes from two ADH cDNA clones from Monterey pine.

Species	No. pfu Screened ($\times 10^6$)	Probe		Positive to Both Probes
		1025	1029	
Monterey Pine	1.0	30	Not Done	NA
	1.5	30	9	3
Loblolly Pine	2.5	77	35	12

Results of such analyses are summarized in Figure 1 for 24 clones of loblolly pine identified using 1025 as a probe. We present here data for SalI/EcoRI double digests because pine DNA contains many recognition sequences for EcoRI and we limit our discussion to insert fragments that contain at least 3 recognition sites for these enzymes (i.e. the insert is cleaved into 4 fragments). In these cases, two of the insert fragments are "full-length". No two of these clones are identical (Figure 1), but this is perhaps not surprising because overlapping clones were intentionally generated by partial digestion with Sau3A in the construction of the library.

Clones containing similar sized restriction fragments should be compared to determine whether they contain overlapping sequences of genomic DNA. To facilitate such analyses, clones were sorted by the length of their hybridizing fragment(s) (Figure 1). Clones designated B and C are the best example of a pair of clones likely to contain overlapping sequences. Both clones contain a 1.4 kb fragment that hybridizes to 1025, and they contain 0.4 and 1.5 kb fragments that do not hybridize. Two other pairs (J-K and M-N) each contain one other similar-sized fragment in addition to the similar-sized hybridizing fragment. From these limited data, there is no convincing evidence that other clones overlap. These results are now being extended by digesting DNA with Sau3A, which has a 4-base recognition sequence and is expected to cleave insert DNA into many smaller fragments.

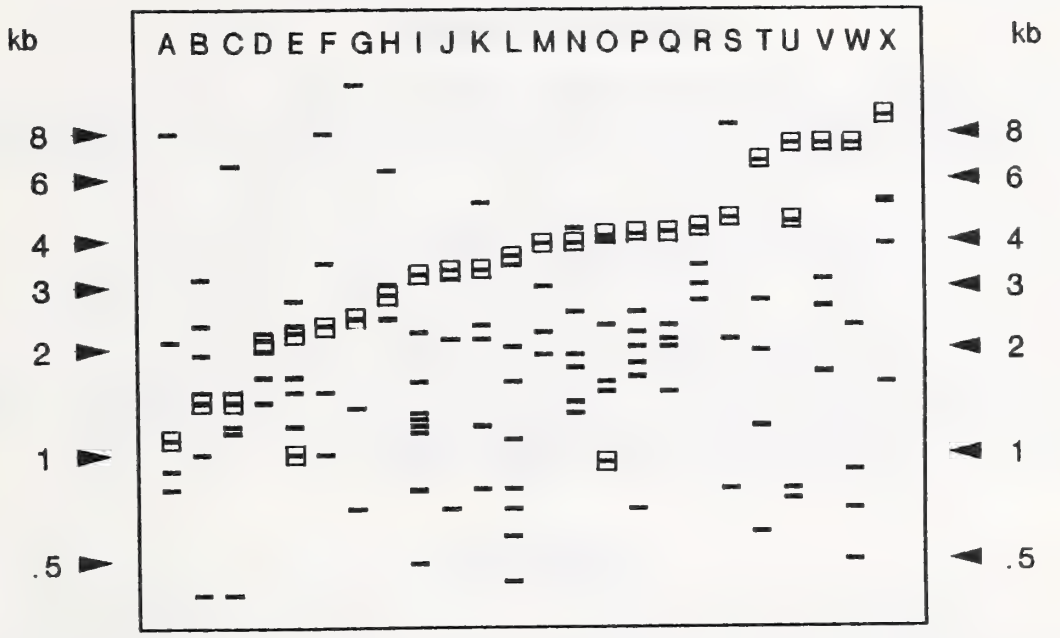

Figure 1. Diagrammatic representation of restriction fragments from genomic clones of loblolly pine, as they would appear on a gel. Shown for each clone are fragment lengths of insert DNA digested with both EcoRI and SalI. Fragments that hybridize to ADH cDNA probes are indicated by a box. Clone ID is coded along the top of each "lane". Arrangement of the clones is by size of the hybridizing fragment. Clones that represent overlapping regions of genomic DNA should share DNA fragments of the same size. Clones B and C probably contain such overlapping genomic DNA.

We are particularly interested in identifying clones that contain an entire ADH gene. The most direct strategy to identify such clones is to determine those clones that hybridize to DNA homologous to regions at both the 5' and 3' ends of the gene. While our pine ADH cDNAs contain sequences near the 5' end of the gene, they do not contain sequences at the 3' end.

As an indirect measure to-date, we have probed blots of the genomic clones with purified insert DNA from the full length maize ADH cDNA pZML793. So far, we have not identified insert fragments that hybridize to the maize cDNA that have not already been observed to hybridize to pine ADH. We are now repeating these experiments with a subclone of pZML793 that represents the 3' portion of the coding sequence that does not overlap with our pine cDNAs.

SUMMARY AND CONCLUSIONS

Pine genomes contain many DNA sequences homologous to the coding sequence of ADH. Many different genomic clones have now been isolated and they appear to represent different portions of the pine genome. Whether any of these clones contain an intact ADH gene is not yet known. We cannot account for the large number of ADH sequences we observe in pines by extrapolating from the structure and organization of ADH genes and gene family in angiosperms. One possibility is that pine ADH genes are large, containing many introns or containing large introns. Another possibility is that the ADH gene family contains several members in addition to those already characterized by isozyme analyses. Whether ADH in pines turns out to be typical of other pine genes remains to be seen. If so, this will have important ramifications for such diverse tasks as gene identification and isolation, and genome analysis and mapping by RFLPs.

ACKNOWLEDGEMENTS

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PRELIMINARY EVIDENCE OF TRANSFORMATION AND FOREIGN GENE EXPRESSION IN SWEETGUM (Liquidambar styraciflua L.) AND LOBLOLLY PINE (Pinus taeda L.)

R.P.Feirer[1] and S.R. Wann[2]

Abstract. Preliminary evidence has been obtained which documents the successful transformation of two commercially important forest tree species. A tumor was produced on an in vitro derived sweetgum shoot inoculated with a binary Agrobacterium vector. Tumor-derived callus displayed growth in the presence of kanamycin, exhibited measurable levels of CAT activity and, as shown by Southern blot analyses, contained DNA originating from the A. tumefaciens binary vector. While no plants have been regenerated from the transformed cells, these findings encourage further work with regenerable sweetgum tissue culture systems. Transformation of loblolly pine was achieved via co-cultivation of an Agrobacterium vector with immature somatic embryos grown in suspension cultures. To date, transient expression of the GUS (ß-glucuronidase) marker gene in intact loblolly pine cells has been demonstrated. The opportunity to transform embryogenic conifer cultures offers many advantages in the genetic transformation and subsequent propagation of economically important softwood species.

Keywords: Liquidambar styraciflua L., Pinus taeda L., Agrobacterium, transformation, CAT, GUS

INTRODUCTION

Assured supplies of fiber for the U.S. pulp and paper industry require reliable silvicultural systems for establishing and managing plantations from genetically improved material. Vegetative propagation and modern recombinant DNA techniques coupled with traditional genetic improvement programs may become part of such a system. Among the first applications of this new technology may be the transfer of foreign genes into genetically superior stock. After testing, the resulting transgenic individuals would then be incorporated into a traditional tree improvement/breeding program. While foreign traits under single-gene control have been successfully transferred into crop plants, progress with forest tree species has not been as rapid, with a notable exception being the transfer of a gene imparting glyphosate tolerance into a Populus hybrid (Filatti, et al., 1987). Clearly, more work is needed on the techniques for the genetic transformation of economically important tree species. An important step in this work is the transfer of marker genes into cells capable of regeneration/propagation in vitro.

In the southeastern United States, sweetgum (Liquidambar styraciflua L.) is a candidate for plantation forestry, as it exhibits fast growth on a wide variety of sites and produces a pulp that blends well with pulp of southern pines. Sweetgum has been propagated in vitro from shoot cultures established from seedling (Sommer et al., 1985) and mature tree explants (Sutter and Barker, 1985). In addition to serving as a system for clonal propagation, shoot cultures also provide a convenient source of sterile plant material to explore gene transfer with Agrobacterium tumefaciens. Using micropropagated shoots, we describe the first successful introduction of a foreign gene into this important southern hardwood forest species.

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Loblolly pine (Pinus taeda L.) is a softwood species of unquestionable importance for the production of fiber in the southeastern United States. The recent achievement of somatic embryogenesis in loblolly pine (Becwar et al., 1989; Gupta and Durzan, 1987), as well as other conifers (Hakman et al., 1985), warrants the initiation of work on the genetic transformation of this species. Transient expression of a marker gene in electroporated loblolly pine protoplasts has been reported (Gupta et al., 1988), but problems related to the regeneration of plants from conifer protoplasts may hinder this approach. Successful infection of loblolly pine seedlings by Agrobacterium tumefaciens has been demonstrated by Sederoff, et al. (1986), suggesting that this biological vector can be used in the transfer of foreign DNA into pine cells. The use of intact cells in this procedure precludes problems related to the consequent regeneration of plants from protoplasts. Reported here are the results of initial attempts to transform cells in embryogenic loblolly pine suspension cultures.

MATERIALS AND METHODS

Transformation of Sweetgum

Small cuttings (8-10 inches long) containing dormant lateral buds were harvested in March. Cuttings were obtained from trees selected in 3-year-old progeny tests (G. Hansen, Union Camp Corporation, Franklin, VA). Buds were picked from the cuttings and soaked for 30 minutes in a solution containing a small amount of surfactant (Tween 20, 0.1%). Buds were sterilized with 10% commercial household bleach (Hilex, 0.525%) for 15 minutes. After three rinses with sterile water, the apical meristems (plus 3-4 primordial leaves) were excised and sterilized with 1% Hilex for five minutes. After three additional rinses with sterile water, shoot tips were placed on WPM medium (Lloyd and McCown, 1980) containing 0.05 mg/l NAA and 1.0 mg/l BA solidified with 0.8% agar (pH = 5.6). Following the procedure of Sutter and Barker (1986), shoot tips were transferred to fresh medium every 3-4 days. Within 3-4 months, each shoot tip had proliferated a number of shoots and, by the end of six months, stable, rapidly growing shoot cultures were established.

The Agrobacterium tumefaciens binary vector used (pGA515-47) was developed and described by An (1986). The oncogenic A. tumefaciens strain A281 carrying the binary vector, which contains neomycin phosphotransferase II (npt II) and chloramphenicol acetyltransferase (CAT) genes driven by nopaline-synthase (nos) promoters, was maintained as described (An, 1986). Shoots, 2-3 cm long, were excised from the cultures and inoculated on the stem. Inoculation was performed using an overnight culture of A. tumefaciens using a 22 gauge needle to make a small wound, into which 10 µl bacterial culture was applied. After 8 weeks a gall was observed on the stem of one of twelve shoots. Sixteen weeks after inoculation, the gall was excised and placed onto shoot proliferation medium (WPM medium with 0.05 mg/l NAA and 1.0 mg/l BA) containing carbenicillin (500 ug/ml) and cefotaxime (250 µg/ml) to kill any remaining Agrobacterium. A pale white callus proliferated from this gall was maintained in culture by monthly transfer to fresh medium (antibiotics not present).

CAT activity was determined by the method of Gorman et al. (1982) employing the modifications of An (1986). Additionally, the plant extract was incubated at 65 °C for 10 min before use in the assay. Plant extract (20 µl) was incubated with 100 µl of reaction buffer, to which 0.1 µCi [^{14}C]-chloramphenicol (54 mCi/mmol, Amersham Corp.) was added. After 30 min incubation at 37 °C, chloramphenicol and the resulting acetylated derivatives were separated on a silica gel TLC plate. The chloramphenicol and radioactive derivatives were then visualized by autoradiography.

DNA was isolated from transformed and untransformed control callus using the method described by Dellaporta et al. (1983). Approximately 3 µg of DNA was digested with Eco R1 according to

manufacturer's instructions and electrophoresed in a 0.7% agarose gel. Undigested DNA from the transformed plant, as well as Eco R1 digested and undigested plasmid DNA (pGA515-47) were also included on the gel. Following electrophoresis, the DNA was transferred to Zeta-Probe nylon blotting membranes (Bio-Rad Laboratories) using an alkaline blotting technique (Read and Mann, 1985). The membranes were then hybridized with radioactive probes consisting of either the pGA515-47 plasmid or the npt II sequence (Church and Gilbert, 1984). Radioactive probes were prepared by nick translating the entire pGA515-47 plasmid or oligo-labeling a Bam H1 fragment (which contains only the npt II sequence) of pDO421. Labeling reactions were carried out according to the manufacturer's instructions. After hybridization, the membranes were washed at 65 °C (Maniatis et al., 1982) and exposed to Kodak XAR-5 film.

Transformation of Loblolly Pine

Embryogenic calli of loblolly pine were initiated from immature embryo explants on MSG medium as described by Becwar et al. (1988; 1989). Suspension cultures were derived by transfer of calli to liquid DCR medium (Gupta and Durzan, 1985) containing 2,4-D (3 mg/l) and BA (0.5 mg/l). These cultures were grown on a gyratory shaker under a 16-hour photoperiod at 24 °C and maintained by transfer to fresh medium every 14 days.

LBA4404, a disarmed Agrobacterium tumefaciens, carrying the binary vector pBI121 was obtained from Clontech Laboratories, Palo Alto, CA. This plasmid vector contains the E. coli derived GUS (beta-glucuronidase) gene joined to a CaMV 35S promoter and a NOS poly-A site (Jefferson et al., 1986). Infection of suspension cultured loblolly pine cells was carried out by adding 100 µl of an overnight culture of Agrobacterium to 25 ml of fresh medium (DCR) containing 1,300 µl (packed cell volume) of plant cells. The 125 ml shake flasks were then incubated at 28 °C on a gyratory shaker. After a 24 hr cocultivation period, plant cells were collected from the flasks, washed with fresh medium (DCR), and placed into 100 mM phosphate buffer (pH = 7.0) containing 100 mM sucrose as an osmoticum and 2 mM 5-bromo-4-chloro-3-indolyl-glucuronide (X-Gluc) (Jefferson, 1987). Although some color development was observed after 1 hr, cells generally had to be incubated in this substrate solution 4 to 6 hr before blue coloration of transformed cells became obvious.

RESULTS AND DISCUSSION

Sweetgum transformation

Approximately 8 weeks after inoculation, a small growth was noted on one of twelve treated shoots. This growth formed a callus and exhibited characteristics of a crown gall phenotype. After eliminating the Agrobacterium with cefotaxime, the callus was maintained on WPM (NAA and BA at 0.05 and 1.0 mg/l, respectively) containing kanamycin at 100 ug/ml. WPM containing NAA and BA at these levels did not typically support normal callus growth of sweetgum. The callus thus exhibited two characteristics of transformed tissue; the ability to grow in the absence or at reduced levels of plant growth regulators and the ability to grow in the presence of kanamycin, a trait imparted by the npt II gene present in the T-DNA. Untransformed control sweetgum callus proved unable to grow in the presence of kanamycin.

Chloramphenicol acetyltransferase activity assays were carried out to demonstrate the presence and expression of the CAT gene in the transformed tissue. Acetylated derivatives of chloramphenicol were formed with extracts of transformed tissue but not untransformed control tissue (data not shown). Although present at measurable levels, the CAT activity in the transformed tissue was very low. Higher levels of CAT expression might have been obtained using the 35S cauliflower mosaic virus promoter, which has been reported to be 10-15 fold stronger than the NOS promoter (Morelli et al., 1985). Insertion of the CAT gene into an inactive region of the genome or the presence of an inhibitor of CAT activity in extracts of the

transformed tissue might also have been responsible for the low CAT activity observed. Extracts of non-transformed control tissue were found to reduce the apparent activity of authentic CAT isolated from E. coli (data not shown), suggesting the presence of such an inhibitor.

The most convincing evidence demonstrating transformation of the tissues was provided by Southern blot analysis. When probed with the entire pGA515-47 plasmid, the probe hybridized to several bands in the lane containing DNA isolated from the transformed tissue (Figure 2a). The lengths of these fragments agree with those expected from an Eco R1 digest of the T-DNA of pGA515-47. The probe did not bind to any fragment of the DNA isolated from untransformed control callus, providing evidence that the probe did not hybridize to a sequence normally present in the sweetgum genome. To prove that the probe was hybridizing to T-DNA that was incorporated into the sweetgum genomic DNA, and not to plasmid DNA that might have been present in Agrobacterium contaminating in the callus tissue, uncut DNA isolated from transformed tissue was probed (Figure 2a, lane 3). The probe hybridized to high molecular weight (undigested plant) DNA. No bands characteristic of those obtained when the probe hybridizes to undigested pGA515-47 plasmid DNA were observed. Thus, hybridization signals in the lanes containing DNA isolated from transformed tissue result from the presence of T-DNA in the sweetgum genomic DNA.

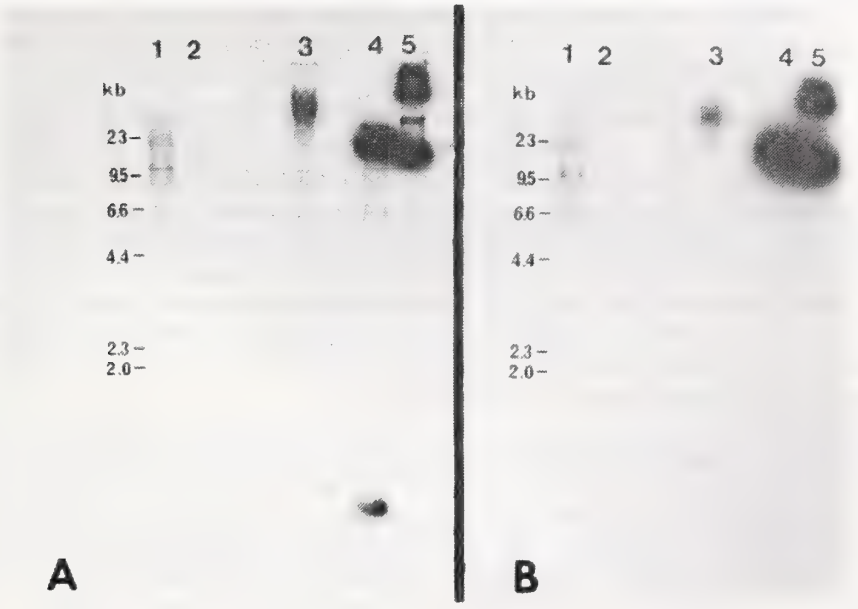

Figure 1. Southern blot analyses of Eco R1 digested DNA isolated from transformed (lane 1) and untransformed (lane 2) sweetgum calli. Lane 3 contained uncut, high molecular weight DNA isolated from transformed callus. Controls included uncut (lane 5) and Eco R1 digested (lane 4) pGA515-47 plasmid. Membranes were probed with nick translated p515-47 (A) and an oligo-labelled npt II gene fragment (B).

A more specific probe was also used to confirm transformation. Using only the npt II sequence as a probe in Southern blot analysis, the presence of the npt II gene was demonstrated in the DNA of the transformed tissue (Figure 2b). Several bands are missing in this blot when compared with the results obtained using the entire Ti-plasmid as a probe, presumably due to hybridization of pGA515-47 to fragments containing the CAT gene or T-DNA border sequences. As shown on the previous blot, the probe

hybridized with high molecular weight plant DNA in the lane containing the uncut DNA from transformed tissue, and did not give a hybridization pattern expected if Agrobacterium were still present in the transformed tissues. This blot again confirms the presence of foreign genes, specifically npt II sequences, in the genome of the transformed sweetgum callus. The apparent ability of the transformed tissue to grow in the presence of kanamycin suggests that the npt II gene is not only present, but is being expressed.

Transformation as well as regeneration of transformed Populus hybrids has been reported (Pythoud et al., 1987; Fillatti et al.,1987; Parsons et al., 1986). The transfer of foreign genes into sweetgum and the subsequent expression of those genes in the transformed tissue are demonstrated in this report. The presence of the foreign DNA was confirmed by Southern blot analyses, and expression of the transferred genes was suggested by tumor formation on the inoculated explant, the ability of the transformed tissue to grow in the presence of kanamycin and measurable levels of CAT activity in extracts of the tissue. That this sweetgum transformation scheme must be further optimized is affirmed by the low frequency of gall formation on inoculated plants. This work may lead, however, to studies of gene expression and transfer of economically important traits in this commercially important hardwood species. In this regard, it is noteworthy that leaf disc regeneration systems that have found extensive application in Agrobacterium-mediated transformations (Horsch, et al., 1985) have been developed for sweetgum (Brand and Lineberger, 1988).

Loblolly pine transformation

After incubation of washed, cocultivated loblolly pine cells in a solution containing the colorless, synthetic substrate X-Gluc, a significant proportion of the cells appeared blue upon examination by brightfield microscopy. While the distribution of the blue color within the cell compartments (i.e. vacuole or cytoplasm exclusively) was difficult to judge, the color was clearly located intracellularly. This was especially evident in those cells that had plasmolyzed, with the dye clearly being limited to the area inside the plasma membrane. Cocultivation periods of 24 and 48 hr were tested, with no apparent improvement in transformation success at 48 hr. Embryogenic conifer calli and suspension cultured cells typically contain two cell types; large, elongated, highly vacuolated "suspensor" cells and smaller (pro)embryonic cells exhibiting dense cytoplasm. In these transformation studies, both cell types exhibited blue coloration, evidence of transient expression of the GUS gene.

Removal of Agrobacterium from the cocultivated plant cells remains a problem. While the bacteria can easily be killed in cocultivated carrot suspensions by the addition of antibiotics to the culture medium (Scott and Draper, 1987; Feirer, 1988), the Agrobacterium persisted in the loblolly pine cultures (similar problems have been encountered with Norway spruce cultures in our laboratory). Consequently, bacterial contamination of the cultures was a problem, complicating attempts to obtain stably transformed plant cell lines. Therefore this report demonstrates only the transient expression of the foreign gene.

Transformation of loblolly pine protoplasts and cells in intact seedlings has previously been reported (Dandekar et al., 1987; Gupta et al., 1988; Sederoff et al., 1986). The work reported in this study differs, however, in the use of Agrobacterium as a vector to deliver foreign DNA to embryogenic, intact cells. Use of intact cells rather than protoplasts should facilitate easier regeneration of transformed plants. Especially notable is the use and apparent transformation of competent cells in embryogenic cultures. Although regeneration of intact plants from embryogenic loblolly pine cultures remains difficult, when the tissue culture techniques are optimized to successfully produce whole plants this transformation technique will be immediately useful. The cocultivation of embryogenic cells has been successfully used in the production of both transgenic carrot cells and intact plants (Scott and Draper, 1987; Feirer, 1988). This technique offers the advantages of high transformation efficiencies, 60 to 70 % (Scott and Draper, 1987) and the ability to use regenerable, easily manipulated cultured cells.

The E. coli derived GUS gene has served as a reliable model gene for use in plant transformation studies with a number of plant species, including coniferous tree species (Jefferson, 1987; Wilson et al., 1989; Zhang and Wu, 1988). Bekkaoui et al. (1988) have recently reported that this marker gene may be unsuitable for use in white spruce cells due to high levels of endogenous compounds which interfere with the fluorometric measurement of GUS activity. These authors, using the marker gene in protoplast electroporation studies, suggested that the interfering compound(s) might possibly be released upon the electroporation process and related lysis of protoplasts. Wilson et al. (1989), reported that background "GUS-like" activity in the fluorometric assay was not a major problem in PEG-mediated transformation of white spruce protoplasts, however. Our results were uncomplicated by high background levels of GUS activity (cells in control cultures remained colorless in presence of x-Gluc), and this may be related to the use of intact cells or the use of the histochemical rather than the fluorometric detection of enzyme activity.

CONCLUSION

While reports of transformation of agronomic crops are plentiful in the literature, work with forest trees has lagged. This report provides evidence for the transformation of two commercially important forest tree species. The use of intact cells, rather than protoplasts, should simplify the regeneration of transformed plants from the shoot cultures of sweetgum and embryogenic loblolly pine cultures. The results of this study also reinforce the earlier observations (Bekkaoui et al., 1988; Wilson et al., 1989) that foreign genes and promoters are functional in conifers, including loblolly pine.

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INTRODUCTION OF FOREIGN DNA INTO YELLOW-POPLAR PROTOPLASTS BY ELECTROPORATION

H.D. Wilde[1], R.B. Meagher[2], and S.A. Merkle[1]

Abstract. Plantlets of yellow-poplar can be regenerated from protoplasts by somatic embryogenesis. This culture system could allow the production of genetically altered trees following DNA uptake into protoplasts. Two bacterial marker genes, β-glucuronidase (GUS) and neomycin phosphotransferase (NPT II), are being used to monitor yellow-poplar transformation. Plasmids bearing one or both of these genes were introduced into yellow-poplar protoplasts by electroporation or polyethylene glycol-mediated uptake. The protoplasts had been isolated from embryogenic suspension cultures at different timepoints after transfer to fresh medium. In addition to the physical parameters of electroporation, such as voltage, capacitance, and pulse length, an important factor in delivery of DNA into cell nuclei was the interval between subculture and exposure to DNA. Assays for GUS activity indicate that the highest level of transient expression was in protoplasts isolated from 20-day old cells.

Keywords: Liriodendron tulipifera, electroporation, protoplasts, polyethylene glycol, transient expression, β-glucuronidase

INTRODUCTION

Gene transfer technology can be applied to forest species for which regeneration systems have been developed. Yellow-poplar (Liriodendron tulipifera) is an ideal candidate for genetic transformation because it can be regenerated from cultured cells by somatic embryogenesis (Merkle and Sommer 1986). Protoplasts from embryogenic suspension cultures will form callus that retains developmental potential (Merkle and Sommer 1987). Methods involving the direct DNA transformation of protoplasts can be used as an alternative to Agrobacterium-mediated transformation. This approach avoids the host-range limitations of Agrobacterium.

Protoplasts can be induced to take up foreign DNA with a brief exposure to a high intensity electric field (electroporation) or polyethylene glycol (PEG). These techniques must be optimized for each species and tissue source. The efficiency of DNA uptake can be measured by the degree of transient expression of a reporter gene. In these studies, the gene encoding β-glucuronidase (GUS) was introduced into yellow-poplar protoplasts and its expression was quantitated by a fluorometric assay (Jefferson et al. 1987).

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METHODS

Protoplast Preparation

Protoplasts were isolated from two embryogenic suspension culture lines by a modification of the procedures of Merkle and Sommer (1987). Briefly, approximately one gram of tissue was placed in 10 ml of filter-sterilized digestion medium containing 1% Cellulysin and 0.5% Macerase. After 24 hours of incubation at 30^0 C, the cells were filtered through Miracloth, washed twice by sedimentation, and sieved through a 25 µm pore stainless steel mesh. The length of time between subculture of the cell suspensions and protoplast preparation was varied. The average number of protoplasts/g after 5-day and 20-day intervals was 6.4×10^6 and 1.7×10^7, respectively.

Transformation of Protoplasts

The protoplast concentration was adjusted to 1×10^6 protoplasts/ml for electroporation and 2×10^6 protoplasts/ml for PEG-mediated transformation. Uncut plasmid DNA was added at a final concentration of 25 µg/ml. Either pBI221, which carries the CaMV 35S/GUS/NOS construct, or pBI121, which carries the NOS/NPTII/NOS construct in addition to the same GUS construct, was used (Jefferson et al. 1987). A GUS construct with a soybean heat shock promoter was a gift from W. A. Ainley, Botany Dept., UGA. Protoplasts were electroporated with a Promega Model 450 in a buffer containing 100 mM NaCl, 4 mM $CaCl_2$, 8 % PEG, and 500 mM mannitol. The voltage, capacitance, and pulse length were varied. The pulse decay constant was calculated as the product of the capacitance and resistance (Fromm et al. 1987). Since the initial voltage (V_0), capacitance (C), and pulse length (t) are set and the voltage remaining after the pulse (V_t) is observed, the resistance (R) could be calculated from the following equation:

$$V_t = V_0 e^{-t/RC}.$$

For PEG-mediated transformation, the protoplast/DNA suspension was mixed with one volume of 40% PEG-6000 and incubated at room temperature for 45 minutes. PEG-treated and electroporated protoplasts were washed with yellow-poplar regeneration medium I (Merkle and Sommer 1987) and incubated for 18 hours at 30^0 C in a thin layer of the same medium. Controls without DNA were always included. Protoplast viability was determined by exclusion of 0.5% Evan's Blue dye. After transformation with pBI121, a fraction of the protoplasts was plated in agarose droplets (Merkle and Sommer 1987) and were selected with 100 µg/ml kanamycin after two weeks.

β-Glucuronidase Assay

Protoplasts were sedimented at 100 x g in 15 ml Falcon tubes for 5 minutes and the pellet was resuspended in 50 µl of GUS extraction medium (Jefferson et al. 1987). The suspension was transferred to eppendorf tubes, homogenized briefly with a glass pestle, and spun for 5 minutes in a microcentrifuge. The supernatant was removed and a 10 µl aliquot was taken for protein quantitation by Bradford assay (Bio-Rad kit procedures). An equal volume of extraction buffer containing 2 mM 4-methyl umbelliferyl glucuronide was added to the protein extracts and incubated at 37^0 C. The reaction was stopped at different timepoints with the addition of 0.5 ml of 0.2 M Na_2CO_3. β-Glucuronidase

hydrolyzes the substrate, producing methyl umbelliferone (MU), which is fluorescent when excited by long-wave UV light. The fluorescence of the samples was quantitated with a Hoefer TKO 100 Mini-Fluorometer and the MU concentration was determined from a standard curve. Background fluorescence, determined from the controls without DNA additions, was subtracted and GUS activity was normalized on a per mg protein basis.

RESULTS AND DISCUSSION

Electroporation Parameters

Sucessful transformation by electroporation requires a degree of membrane permeablization that balances gene transfer efficiency with cell viability. Generally, this involves electroporation conditions that have a decay time constant of 5-15 ms and lead to a reduction of 50% in protoplast viability (Fromm et al. 1987). The decay time constant is a function of the capacitance of the electroporator, the voltage to which it is charged, and the resistance of the buffer containing the protoplasts.

Figure 1 shows the effect of field strength (V/cm) on protoplast viability. With a capacitance of 450 microfarads (μF), cell viability was reduced to 24% when a field strength of 800 V/cm was applied. A capacitance of 450 μF was used in further experiments because the higher capacitance did not reduce viability significantly. With a field strength of 700 V/cm, protoplast viability was 53%, which is nearly optimal. The resistance of the electroporation buffer, determined by its ionic strength, was held constant. When the pulse length was set to 30 ms, and with a capacitance of 450 μF and a field strength of 700 V/cm, the decay time constant was calculated to be 12 ms.

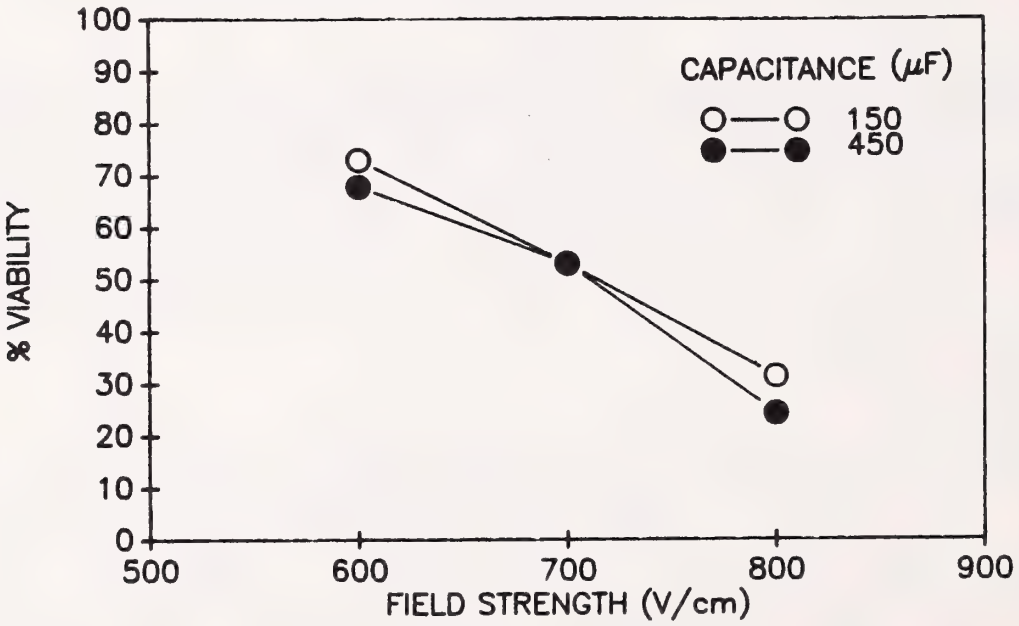

Figure 1. Effect of field strength on protoplast viability. Protoplasts were electroporated and viability was assessed the following day by dye exclusion.

At a field strength of 600 V/cm, protoplast viability remained near 70%. It is typical for small, embryogenic cells to require higher field strengths for membrane permeabilization than larger, vacuolated cells from, for example, mesophyll tissue. Yellow-poplar protoplasts isolated from embryogenic cell suspensions are small (10-20 μm diameter) and densely cytoplasmic. Electroporation conditions for these protoplasts are comparable to those for other protoplasts with regenerative potential. Table 1 shows electroporation parameters for yellow-poplar, rice (Toriyama et al. 1988), carrot (Fromm et al. 1985), and maize (Fromm et al. 1986).

Table 1. Electroporation parameters of protoplasts from suspension cultures with regenerative potential.

	Field strength	Capacitance	Decay constant
yellow-poplar	700 V/cm	450 μF	12 ms
rice	750	22	4
carrot	875	980	20
maize	500	245	4

Influence of culture cycle on transient expression

Yellow-poplar suspension cultures were subcultured into fresh medium every two weeks. Protoplasts were routinely isolated one week after subculture, but GUS expression was never detected after these protoplasts were electroporated with either pBI121 or pBI221. GUS was expressed transiently, however, in protoplasts isolated two and three weeks after subculture. To examine this variation in more detail, protoplasts were isolated at five-day intervals after a subculture and were incubated with pBI221 and 20% PEG. After washing and overnight incubation, the protoplasts were lysed and assayed for GUS activity. The results are shown in Figure 2.

PEG-mediated transformation of protoplasts isolated from culture line TP 14x108 at 5, 10, and 25 days after subculture produced low levels of GUS activity. The identical treatment of protoplasts isolated 15 and 20 days after subculture led to a 2-fold and 6-fold increase, respectively, in GUS activity. A similar, though less well pronounced, pattern of GUS expression was evident in protoplasts from tissue culture line TP 4x12. This suggests that the physiological age of the protoplasts may affect their ability to take up or express foreign DNA. The differential expression of the chaemeric gene does not appear to be related to the CaMV 35S promoter, because the same results were observed when a soybean heat shock promoter-GUS construct was used. Transformation efficiency was found to be affected by the mitotic state of the protoplasts in tobacco (Meyer et al. 1985, Okada et al. 1986). Yellow-poplar suspension cultures are asynchronous, however, so the contribution of a single mitotic state would be minimal.

When the PEG concentration was increased from 8 to 20%, PEG-mediated DNA uptake lead to levels of GUS activity in the range previously obtained by electroporation. This method of transformation was less deleterious to

protoplasts, as evidenced by improved colony production when these protoplasts were plated in agarose droplets. Kanamycin (100 µg/ml) was used to select colonies derived from protoplasts after PEG-mediated uptake of pBI121. This plasmid carries a gene encoding neomycin phosphotransferase II, which confers antibiotic resistance. While cells not exposed to pBI121 died, some colonies which had been treated with the plasmid survived. Similarly treated protoplasts from the same experiment showed transient GUS activity. Transformation of these colonies cannot be confirmed until DNA analysis has been completed.

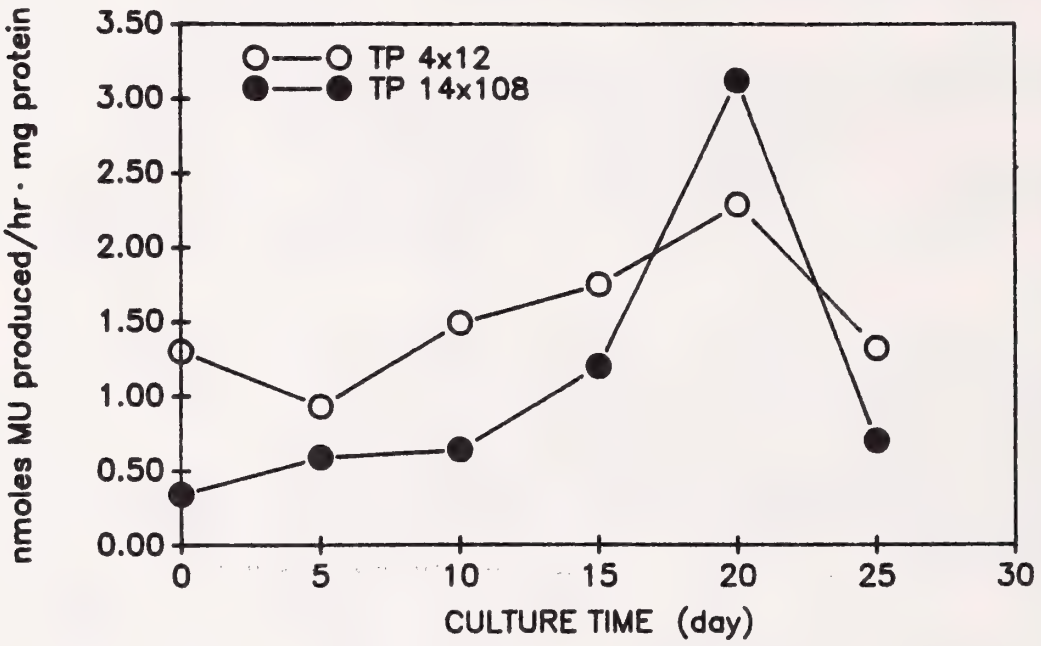

Figure 2. Transient GUS expression in protoplasts isolated at different timepoints after subculture. GUS activity shown is the average of two transformations per timepoint per experiment. The experiment was repeated twice for each culture line.

CONCLUSIONS

Plasmid DNA was introduced into yellow-poplar protoplasts by both electroporation and PEG-mediated uptake. Electroporation parameters were adjusted to give a pulse decay constant of 12 ms and protoplast viability of 53%. Conditions for electroporation of yellow-poplar protoplasts were in the range of those for small, developmentally active protoplasts from other species. Transient expression of the GUS gene showed that DNA uptake by PEG treatment alone was as effective as electroporation when the PEG concentration was raised to 20%. The physiological age of the protoplasts influenced the amount of GUS activity observed after the uptake of pBI221. Whether protoplasts at various stages of the culture cycle differ in the ability to take up or express the DNA is unknown. The introduction of an antibiotic gene from pBI121 has apparently permitted some colonies to survive on 100 µg/ml kanamycin.

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GENERAL SESSION:
TREE IMPROVEMENT PROGRAMS

A GENETIC IMPROVEMENT PROGRAM FOR VIRGINIA PINE CHRISTMAS TREES

C. R. McKinley [1/]

Abstract.--In 1981, a cooperative effort was initiated to provide genetically improved Virginia pine seedlings to Christmas tree producers in the state of Texas. A total of 50 parental selections were evaluated in four genetic tests located in east Texas. Concurrent with the establishment of genetic tests was the planting of a ten acre seedling seed orchard.

The orchard has been rogued once on the basis of results obtained from genetic tests and first seed production was realized in 1987. As seed production increases additional roguing is anticipated.

Second-generation selections have been made using criteria suitable for evaluation of Christmas trees. These selections have been preserved by grafting into a scion bank. A breeding program utilizing the concept of matings within breeding groups has been developed to provide for additional genetic gain in future generations.

Keywords: Christmas trees, Pinus virginiana Mill.

INTRODUCTION

The production of Christmas trees has become a substantial industry in the state of Texas with approximately one million Virginia pine (Pinus virginiana Mill.) seedlings being planted annually. Because of the potential for loss of the existing seedling supply and a desire to achieve as much genetic improvement as practical, the Texas Christmas Tree Grower's Association (TCTGA) expressed a desire to develop an on-going tree improvement program for Virginia pine selected for use as Christmas trees. Thus, in October, 1981 the Virginia Pine Christmas Tree Improvement Program was formally begun.

ORGANIZATION

The Virginia pine improvement program is a cooperative effort among the TCTGA, the Texas Agricultural Extension Service (TAEX) and the Texas Forest

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[1/] Associate Geneticist, Texas Forest Service, Texas A&M University System, College Station, Texas.

Service (TFS). The grower's association provides monetary support, the TAEX provides technical assistance and the TFS is responsible for tree improvement activities. The cooperative is guided by a steering committee composed of representatives of the three organizations which meets periodically to review program status and activities.

While the TFS is responsible for seed orchard production, one-half of all seed produced is made available to the grower's association for the cost of cone harvest and seed extraction. Nursery contracting for growing of TCTGA seedlings remains the option of that organization. The remaining one-half of the seed is retained by TFS to be grown for open-market sales.

TREE IMPROVEMENT PROGRAM

The Virginia pine improvement program follows many of the same procedures utilized by other southern pine improvement programs (Figure 1) and consists of four principal components: 1) selection, 2) testing, 3) orchard establishment and management and 4) breeding.

Selection

Selection of parental material was accomplished from within the native range of Virginia pine using two procedures.

1. Selection from within native stands

Forty trees were selected from native stands in central Alabama and cones collected in fall, 1981. Although straightness, apparent growth rate and general appearance were considered, the primary selection criteria was the presence of cones from which to extract seed. An effort was made to select parents which were geographically distributed sufficiently such that related individuals were not chosen.

2. Selection of previously tested material

Trees in progeny tests belonging to several industrial and state organizations were inspected and evaluated for potential merit as Christmas trees. Following evaluation, open-pollinated seeds from the best families were obtained from seed orchard ramets. Seeds from a total of 15 parents from three orchards were obtained in this manner. Because orchard seed was involved, some relatedness due to the pollen source was possible.

Testing

Following cone extraction, seeds from 50 of the selected individuals were stratified and planted into the Texas Forest Service nursery near Alto, Texas. Seedlings were grown for one year using standard nursery practices except that no undercutting, lateral pruning or top pruning was done.

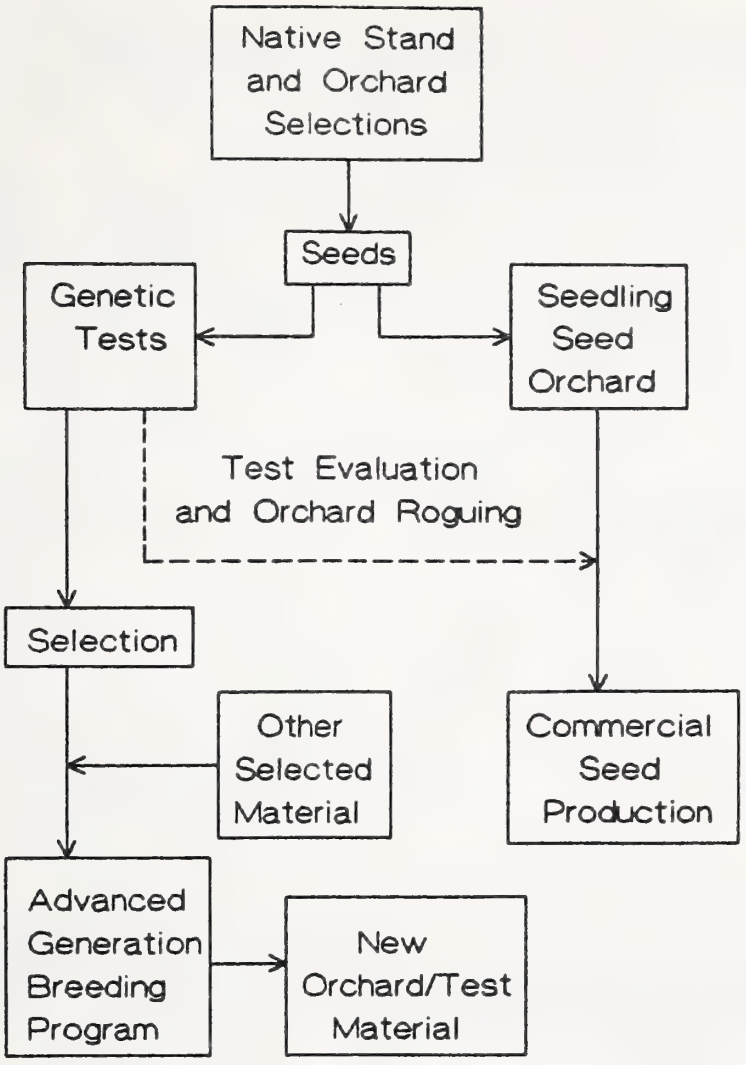

Figure 1. Diagram of major activities associated with Virginia pine improvement program.

Seedlings were lifted in March, 1983 and planted into four progeny tests in east Texas (Figure 2). Planting sites were located from near the gulf coast to the northeast corner of the state. Each planting consisted of six replications with eight trees per replication/family.

Genetic tests were established on lands belonging to private Christmas tree growers with the agreement that the tests be maintained until commercial harvest. No specific instructions were provided these growers other than to manage the tests as operational plantings with any treatments be applied uniformly.

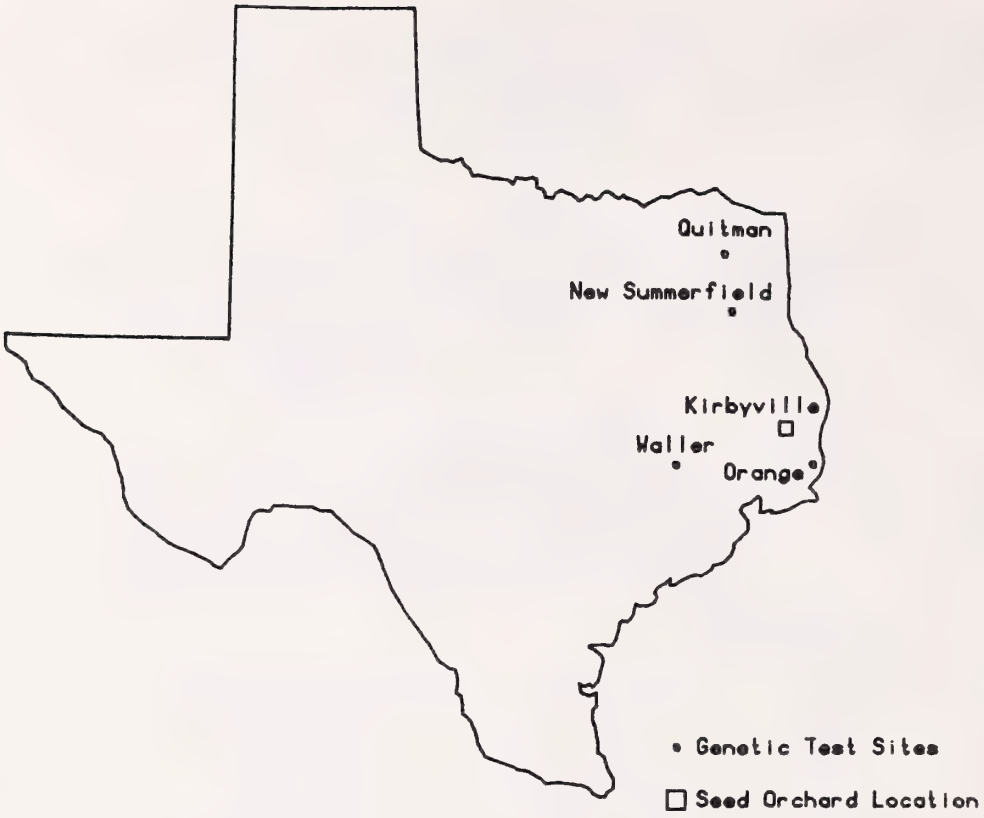

Figure 2. Location of genetic tests and seed orchard for Virginia pine improvement program.

Test measurements (Table 1) consisted of first-year survival and height, second-year height and third-year height. After the second growing season, seedlings were also evaluated for number of whorls, straightness and potential for Christmas trees. The latter evaluation is referred to as grade, although it does not represent the commercial grade often used for marketing. Statistical analysis indicated significant ($P<.05$) family differences for all traits within each location with relatively small family by location effects. Using the information obtained, a selection index was developed for use in determining the best families. Individuals within each test were assigned a value based on this index and the highest ranking families across all tests were identified. Orchard roguing utilized the results of this ranking. While several traits were considered, second-year height had the greatest impact on which families were selected.

The tests were released to the cooperating growers after selection of advanced-generation material which was the end of the fourth growing season.

Table 1. Summary of Virginia pine planting means for four genetic tests in East Texas.

	Location			
	Orange	Waller	New Summerfield	Quitman
Number of Sources	48	51	51	44
First-Year Survival (%)	78.9	96.1	98.0	57.7
First-Year Height (cm)	38.2	38.5	45.9	26.3
Second-Year Data				
Height (cm)	98.5	65.6	98.4	67.7
Straightness (0,1)[1/]	.14	.13	.38	.19
Grade (0-4)[1/]	1.98	1.34	1.89	1.93
No. whorls	4.69	4.11	6.14	4.12
Third-Year Height (cm)	122.7	96.0	147.3	117.5

[1/] Higher values represent better scores.

Orchard Establishment and Management

Concurrent with the establishment of the four genetic tests, a ten-acre seedling seed orchard was established near Kirbyville, Texas. Initial spacing was 8'x 14' with either one or two seedlings planted at each location. Seedlings from each of the 50 original selections were planted in a design to minimize the likelihood of potentially related individuals being adjacent to each other.

The orchard was rogued in 1985 to the best 25 families following second-year evaluation of genetic tests. In winter 1986, approximately 300 trees were transplanted within the orchard to provide more uniform spacing. The current spacing is 24' x 28' leading to approximately 65 trees per acre.

The orchard has been managed similar to other southern pine seed orchards although with Virginia pine, greater attention must be paid to control of tip moth (Rhyacionia spp.). Insecticide treatments early in the life of the orchard proved quite beneficial. Likewise, seed and cone insect control has been intensified as cone production has increased.

First seed production was realized in 1987 with nine pounds being produced for the entire orchard. In 1988, 35 pounds of seed was produced from 45 bushels. At age ten, it is projected that 8-10 pounds of seed per orchard acre will be produced. Additional roguing is anticipated as seed production increases. While timing is not yet specifically determined, the

orchard will be placed on an advancing-front schedule relative to removal and re-establishment. This schedule will provide for incorporation of improved material while maintaining a required level of seed production.

Breeding

In fall 1986, the best 25 families were identified on the basis of third-year height in two of the four genetic tests. The other two tests were deemed unsuitable for use based on number of existing trees. Selection of the best individual from within each of these families was then made using criteria applicable for Christmas trees (height, straightness, grade and general appearance). These selections were first grafted into a scion bank in 1987 with additional grafts made in 1988 and 1989. Approximately 45 of these selections remain preserved as some selections were lost due to initial grafting mortality. Of the original 50 families, 37 were represented in the advanced-generation population.

Although not yet initiated, an advanced-generation breeding program is proposed utilizing 100 selections distributed among 10 breeding groups (Figure 3). Included in the 100 selections would be the 45 selections from genetic tests as well as material obtained from other organizations working in Christmas tree improvement. Additionally, several trees of selected families located in the seed orchard can be utilized to replace those selections lost by graft failure.

Control pollination within groups is proposed utilizing a partial diallel mating design. Progeny would then be established in several tests (8-10) throughout east Texas for purposes of evaluation and advanced-generation selection. Evaluation and selection procedures are expected to be similar to those used in the first generation. Selection within each of these tests would be accomplished in order to reconstitute each of the ten breeding groups with 10 to 12 individuals per group, leading to a perpetuating system. New orchards would be established using seed from the best control-pollinated families.

SUMMARY

The Virginia pine improvement program was established to meet the seedling needs for Christmas tree growers in the state of Texas. To date, first-generation selection, genetic testing, orchard establishment, and advanced generation selection have been accomplished. The seed orchard has been rogued once and has provided for commercial seedlings for the past two years.

The next challenge is to initiate a systematic breeding program utilizing the advanced-generation selections, and to establish progeny tests of this material for parental evaluation and future selection.

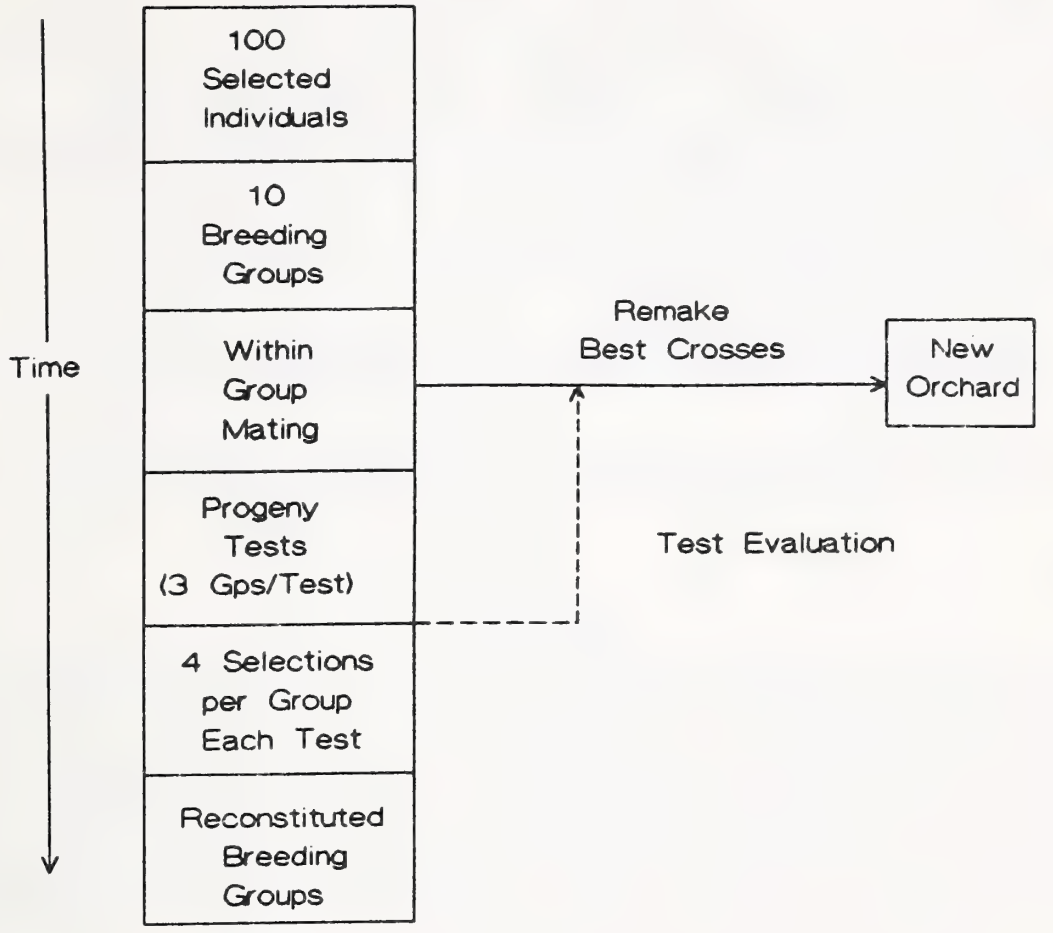

Figure 3. Proposed breeding and selection scheme for advanced-generation material.

ACKNOWLEDGEMENT

The Virginia Pine Christmas Tree Improvement Program has proven to be a most successful venture. Contributing to this success has been the interest and assistance of numerous organizations and individuals. Their efforts on behalf of this program are very much appreciated. A special word of appreciation is due Mr. Jim Chandler, Texas Agricultural Extension Service, and the following growers: Brockett/Tyree Farms, Fangue Farm, K & K Evergreen Christmas Tree Farm, and Tennison Nursery and Christmas Tree Farm.

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GENETIC IMPROVEMENT OF EUCALYPTUS GRANDIS FOR SOUTHERN FLORIDA

D. L. Rockwood, E. E. Warrag, K. Javenshir, and K. Kratz 1/

Abstract.--Genetic gain potentials for a 5th-generation seedling seed orchard were 27 and 17% for 15-month height and survival, respectively. Larger gains may be expected from clonal selection and testing, and 232 clones now under test may have sufficient freeze resilience. Several clones averaged over 6 m tall in 1.25 years on a well-prepared site; three clones performed superbly in two tests. Laboratory freeze screening at -5°C rated clones similar to field results. Early plantlet development was like that of seedlings, and rooted cuttings and plantlets grew well in the field. Large numbers of rooted cuttings can be produced from selected clones, and direct micropropagation of certain clones has been accomplished commercially.

Keywords: Eucalyptus grandis Hill ex Maid., seedling seed orchard, clonal forestry, freeze resilience.

INTRODUCTION

Eucalyptus grandis was planted for pulpwood production in southern Florida from the 1960's to the early 1980's. Concurrently, a genetic improvement program conducted by the U. S. Forest Service increased tree vigor and quality considerably (Geary et al. 1983, Meskimen 1983). Severe freezes in January 1982, December 1983, and January 1985, extreme windborne/inversion freezes as low as -11°C and lasting for up to 18+ hours, provided unique opportunities to develop freeze-resilient, fast-growing trees. Recent genetic improvement activities have emphasized seed orchard development and clonal selection/propagation in support of a modest commercial planting program.

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METHODS

GP77, a 4th-generation genetic base population established July 1977 near LaBelle with 31,725 trees from 529 progenies (Meskimen 1983), was converted to seedling seed orchard GO77 in 1986 (Reddy et al. 1986). Open-pollinated seed was collected in March 1987 from 21 of the best trees. Within a 25 ha test established near Palmdale in August 1982 with progenies from eight superior trees in the 3rd-generation orchard GO73, 232 cloning candidates were located in December 1986 and propagated as rooted cuttings. These and other clones and progenies (Table 1) were outplanted in study ORNL-40 in August 1987 in the Palmdale-LaBelle area. Within a randomized complete block design with three replications, subplots were used for 1) clonal testing, 2) progeny testing, and 3) yield estimation. Clonal test subplots were subdivided into seven sets, each typically containing 4-tree row plots of 37 new clones and three clones 2798, 2814, and 2817 determined to be superior by Meskimen et al. (1987). Progeny test subplots consisted of 10-tree row plots of four progenies from GO73, ramets and progenies of GO77 ortets 2798, 2805, 2814, and 2817, and 17 other GO77 progenies. Yield subplots were 16 24-tree blocks (four rows of six trees) consisting of pure plots of ramets or progenies of 2798, 2805, 2814, and 2817 and mixed plots of various clonal and progeny pairs. Within-row spacing was 1 m; between-row spacing was 2.85, 2, and 1 m, respectively, in the three replications. Height, DBH, and survival data were last taken in October 1988.

Narrow-sense and broad-sense heritabilities and genetic gains were calculated for 1.25-year height and survival using variance components derived by SAS. Survival analyses followed procedures outlined by Becker and Marsden (1972). Genetic gains for an open-pollinated seedling seed orchard with progeny testing and for clonal selection and testing were based on methods presented by Shelbourne (1969) assuming 10,000 trees were outplanted, i. e., 20 seedlings or ramets for each of 500 progenies or clones, respectively.

Table 1.--*Eucalyptus grandis* clones/progenies in ORNL-40 clonal test, progeny test, and yield test components by orchard origin and generation of selection.

Orchard Origin - Generation of Selection	ORNL-40		
	Clonal Test	Progeny Test	Yield Test
GO73 - 1	1499, 1535[1]		
- 2	888, 1038	847, 1038, 1070	
- 3	997, 1001, 1003 1010	1010	
GO77 - 1		3674	
- 2	2814, 2817	2814, 2817, 3336 3424, 3698, 4249 4292, 4305, 4361	2814, 2817 4305
- 3		3516, 3521, 3706 4161, 4268, 4304	
- 4	2798, 2805	2798, 2805, 4157 4160, 4360	2798, 2805

[1]Accession code of ortet/mother tree

Preliminary laboratory freeze screening of E. grandis clones 2786, 2788, 2798, 2805, 2814, and 2817 and E. camaldulensis clone 174 was conducted in October 1988. Up to five approximately six-month-old tissue culture plantlets, rooted cuttings, and seedlings (for E. grandis ortets 2798 and 2814 only) per clone were subjected to five hours of -3, -5, or -7°C during a 24-hour period beginning and ending at 4°C. Response was evaluated after two weeks as percent of leaves retained and after four weeks as percent of prefreeze stem height alive and as percent survival.

Growth and physiological response of plantlets produced by direct micropropagation of clones 2798, 2814, and 2817 (Rockwood et al. 1988) were compared to that of their half-sib seedlings through five months under greenhouse conditions. Hardened plantlets and germinated seedlings were moved from mist to the greenhouse at the same time, and base line dry matter data were collected. Over four harvests at 40 day intervals, plantlets were then compared to seedlings for: survival, net photosynthesis per dry weight, chlorophyll concentrations, total nitrogen percent, dry matter allocation and relative distribution, and relative growth rate.

Large-scale micropropagation of superior E. grandis clones was conducted in 1988 and 1989. In 1988, approximately 40,000 propagules of 11 clones including 2798, 2814, and 2817 were produced using media and procedures described by Rockwood et al. (1988). In 1989, similar procedures were used to produce 40,000 propagules of clones 174, 2798, 2805, 2814, 2817, and 5000. After three weeks in rooting, 20,000 plantlets were transferred to containers to develop and harden for 12 weeks prior to outplanting in July.

RESULTS AND DISCUSSION

Through 1.25 years, differences among the three reps (planting sites) in ORNL-40 vividly demonstrated the influence of site preparation (Table 2). On the site that had very thorough disking and high beds, clones 2798, 2814, and 2817, for example, averaged 93% survival and 5.4 m tall. On the site with low beds, their survival was also 93%, but their height was only 2.0 m, while on the third site, which had herbaceous competition, their survival dropped to 55% and height to 1.6 m. Good site preparation, specifically preplanting application of ground rock phosphate (Geary et al. 1983), bedding, and thorough vegetation control during the first year, is essential to realizing the growth potential of improved E. grandis.

At 1.25 years, progenies derived from 21 of the top-ranked trees in GO77, the 4th-generation orchard, were similar to four of the best progenies from the preceeding orchard GO73 (Table 2). As these GO73 progenies have been evaluated in four to seven other tests, the comparatively good height and survival of the GO77 progenies in their first test is encouraging. In terms of selection generation (Table 1), the 21 GO77 progenies averaged 2.71 and the GO73 progenies 2.25.

Table 2.--Average height, DBH, and survival of Eucalyptus grandis entries in ORNL-40 on the best site and across three sites at 1.25 years.

Study Component - Genetic Entry	Best Site			All Three Sites	
	Height (m)	DBH (cm)	Survival (%)	Height (m)	Survival (%)
Clonal Test-					
Clones 2798, 2814, 2817	5.4	3.8	93	3.2a[1]	81a
232 New Clones	4.3	3.3	65	2.6b*	55b*
Progeny Test-					
4 GO73 progenies	3.0	2.3	98	2.1a	73a
21 GO77 progenies	3.2	2.5	94	2.2a	66a
4 GO77 progenies	4.0	3.1	88	2.6a	62a
4 Clones	3.4	2.3	85	2.3a	69a
Yield Test-					
4 Progenies - Pure Plots	3.4	2.4	79	1.4b	61a
- Mix Plots	4.2	2.8	88	1.6a	60a
4 Clones - Pure Plots	4.9	3.2	82	1.7a	63a
- Mix Plots	5.0	3.3	83	1.7a	68a

[1]Genetic entry means not sharing the same letter within a study component are significantly different at the 5% level.
*Variability among genetic entries significant at the 5% level.

Other comparisons based on generation of improvement suggest that GO77 should produce fast-growing, somewhat frost-resilient trees. GO77 has an average selection generation of 2.77 and contains 296 4th-generation selections. Meskimen (1983) reported large improvements with generation of selection. In GP77 at 64-months, differences between all 1st- and 2nd-, 2nd- and 3rd-, and 3rd- and 4th-generation trees averaged 206%, 20%, and 55%, respectively, for coppice stem volume, and 4th-generation progenies were best in frost resilience and coppice quality (Reddy et al. 1986). Estimated genetic gains in coppice stem volume of seedlings derived from GO77 (compared to the GP77 mean) ranged from 54% if seed were collected from all 1309 orchard trees to as high as 179% if seed was obtained from only the best tree in each of the top 50 progenies (374 progenies retained).

A 5th-generation seedling seed orchard developed by the strategy espoused by Franklin (1986) may continue the improvement in growth that has accrued from this low-cost, short-generation, wide genetic base program (Table 3). While differences among the GO77 progenies were not significant at the 5% level, individual (Table 3) and progeny mean (h^2 = .332) heritabilities and levels of variation resulted in an estimated gain in tree height of 27% above the 4th-generation. These heritabilities, low compared to the estimates of .29 to .63 reported by Reddy (1985) for coppice height, may reflect a higher level of genotype x environment interaction in ORNL-40. Predicted gain in survival was modest. Consequently, commercial seed collection from the best tree in each of the top 50 progenies in a new orchard could be expected to produce faster-growing seedlings than can now be produced by GO77.

Table 3.--Heritabilities and genetic gains in 1.25-year height and survival for seedling and clonal propagation options.

Propagation Option-Estimation Basis	Height		Survival	
	h^2	Gain[1] (%)	h^2	Gain (%)
Seedling - 21 GO77 Progenies	.241	27.1	.092	17.1
Clonal- Six Clonal Sets	.096 to .368	22.2 to 67.6	.020 to .104	4.2 to 27.5

[1]Relative to mean for test component providing heritability estimate

Seedlings from GO77 ortets were comparable to their corresponding clones in one test component but worse in another (Table 2). In 10-tree row plots, four GO77 progenies were slightly taller with lower survival than the rooted cuttings. However, in 24-tree blocks in the yield test component, clones in pure plots had better height and somewhat higher survival than progenies in pure plots. There were also suggestions in the yield test that within plot variability for clones was less than that of seedlings.

Among clone variability was evident in contrasting proven clones with new selections and within the new candidates in the clonal test (Table 2). Clones 2798, 2814, and 2817 grew and survived well. In comparison to these proven clones, the 232 new cloning candidates were shorter and survived poorly overall, reflecting generally less extensive root systems, but varied widely, suggesting selection opportunities for vigor and survival. In comparison to the proven clones, 29 new clones were better in height, and 24 new clones had higher survival. Three clones averaged over 6 m on the well-prepared site after 15 months, and in May 1989 individual trees were over 8 m tall.

Clonal variation was heritable, and larger gains were predicted for propagation of the 50 best tested clones than for seedling production (Table 3). Generally, broad-sense heritablilities exceeded narrow-sense estimates for tree height, indicating that non-additive variation is important for growth improvement. Clonal testing following selection enhanced genetic gain in tree height but had little effect on survival improvement.

Heritabilities and gains calculated from ORNL-40 clones were less than those estimated by Meskimen et al. (1987). As with the seedling estimates, genotype x environment interaction, approximately 26% of phenotypic variance, is a likely cause for the lower values. As observed in ORNL-40, Meskimen et al. (1987) noted that increases in tree size, as well as in frost resilience, would be greater if clonal candidates were tested. These gains in volume were nearly double estimates developed for seedlings from GO77 (Reddy et al. 1986).

Clonal selection and testing then is the best short-term alternative for increasing tree size and developing sufficient frost-resilience. Several options are available for expanding the number of cloning candidates from the current 340 to over 1,000 (Meskimen et al. 1987). The 232 new clones in ORNL-40, whose ortets had minimal stem damage and magnificent freeze-resilience after the

December 1983 and January 1985 freezes, were generally undamaged by a -6°C inversion freeze in February 1989. The uncertainty of testing clones by unpredictable natural freezes, however, necessitates the development of an artificial system of freeze evaluation as an adjunct to field testing.

Laboratory freeze screening results varied with temperature and clone (Table 4). The -3°C minimum was least damaging while the -7°C regime was most severe, but neither temperature caused much differential response among E. grandis clones. However, E. camaldulensis clone 174, excellent in field freeze resilience, surpassed all E. grandis clones in survival at -7°C. Five of six E. grandis clones survived -5°C, and the survival percentages of the six at -5°C paralleled (r = .65) field resilience observed by Meskimen et al. (1987). Notably, some seedlings from clones 2798 and 2814 were hardier than ramets from the same clones.

Table 4.--Responses of seven Eucalyptus clones to three temperature regimes in a laboratory freeze test.

Temperature-Clone	Freeze Response: Two-week Leaf Retention (%)	Four-week Live Stem Height (%)	Four-week Survival (%)
-3°C - All	77 A[1]	95.1 A	97.8 A
-5°C - 174	0 a	1.2 b	100.0 a
- 2786	0 a	1.0 b	50.0 ab
- 2788	0 a	0.0 b	0.0 b
- 2798	0 a	2.1 b	87.5 a
- 2805	0 a	0.6 b	20.0 b
- 2814	25 a	30.0 a	75.0 a
- 2817	0 a	0.6 b	18.2 b
- All	4 B	6.4 B	50.0 B
-7°C - All	0 B	.1 B	12.8 C

[1]Overall means or clone means not sharing the same upper-case letter or lower-case letter, respectively, are significantly different at the 5% level

Micropropagation of proven clones can be an alternative to rooting of cuttings if plantlets grow normally. Development of plantlets produced by direct enforcement of buds from clones 2798, 2814, and 2817 was generally similar to that of seedlings in the greenhouse, with survivals of 98% and 95%, respectively. Plantlets had significantly lower net photosynthesis, chlorophyll a and b concentrations, and total nitrogen percent than seedlings after 40 days in the greenhouse; however, no differences were observed in later harvests. Photosynthesis was correlated more with foliar nitrogen than chlorophyll concentration. Plantlet dry matter accumulation, relative distribution, and relative growth rates differed significantly from seedling values over harvests, but the differences decreased sharply over time. Plantlets had higher shoot and root dry matter and higher root:shoot and relative growth rates than seedlings. The higher root:shoot ratio may have contributed to the high survival observed. Plantlet and seedling trends with harvests indicated similar basic responses to

changing environment.

Genetic differences, i. e., among plantlet clones and among seedling families, were only significant for root and shoot dry matter accumulation and their ratios. Rankings by genetic origin across plantlets and seedlings suggested genetic control independent of propagation method. Through four months in a field test, plantlets and seedlings from these same clones have shown similar trends.

The E. grandis clones used commercially also differed in their response to direct micropropagation. In 1988, clones 2798, 2805, 2814, and 2817 all had adequate multiplication rates. However, their relative sensitivity to cytokinin ranged from 2814 (most) to 2805 (least). Production of apical meristem callus in conjunction with tip necrosis was noted in 2798 and some minor clones. In the greenhouse, all clones were susceptible to an unexpected Cylindrocladium scoparium infection, which halved the number of plantlets available for transfer.

The problems encountered in 1988 were resolved in 1989. Tip necrosis was avoided by using a shorter transfer cycle and careful selection of material. Rooting was higher after altering nutrient concentrations in the rooting medium, and survival following transfer to the greenhouse increased. The container used for growing the outplantable tree was very suitable for good root system development. No infection was incurred.

These commercial efforts have suggested that the cost of a plantlet rooted for transfer to the greenhouse could be as low as $.15. Further progress with growth regulators in production and rooting and with methods for establishing propagules ex vitro will be needed to reach this goal, however. Additional cost reduction may be realized by using plantlets as greenhouse stock plants for hedging. Clonal micropropagation is currently more expensive than rooted cuttings, at an estimated cost of over $.11/cutting, and seedlings.

Genetic, silvicultural, and propagation potentials with E. grandis are promising for its production in southern Florida. Currently, its wood is in strong demand for mulch. It is one of several Eucalyptus species equal to native hardwoods for common pulping processes (Franklin 1977), is also suitable for hydrolysis and for the ester pulping process, and varies genetically in stemwood specific gravity (Rockwood et al. 1988, Wang et al. 1984). Under present economic and production scenarios, E. grandis seedlings and cuttings can be profitably grown in short rotations for energy wood (Rockwood and Dippon 1989).

CONCLUSIONS

Superior progeny-tested trees in GO77 may provide commercial seed for vigorous, somewhat frost-resilient E. grandis. Testing of cloning candidates can result in greater increases in tree size, and clones presently under test may have excellent freeze-resilience. Three clones now recommended for commercial propagation will greatly reduce the freeze risks associated with E. grandis culture in southern Florida. Propagation by rooting and tissue culture is biologically feasible and economically promising. Plantlets survived well under greenhouse conditions and had growth and physiological trends similar to seedlings.

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GENETIC IMPROVEMENT OF PINES IN THE STATES OF CHIHUAHUA AND DURANGO, MEXICO

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Abstract.--Tree improvement programs in Chihuahua and Durango began in 1987 and 1986, respectively. Priority species are Pinus arizonica, P. durangensis, and P. engelmannii plus, in Durango, P. cooperi and as lower priority, P. herrerae and P. teocote. Based on annual precipitation Chihuahua has been divided into 3 regions and 11 seed zones and Durango has been divided into 5 regions and 17 zones. Eleven of the 30 seed production areas planned for Chihuahua have been established so far. In Chihuahua we plan to select 1000 superior phenotypes of each species and selection work is underway. Clonal orchards will be established with 30-50 of the best selections from each seed zone. All selections will be progeny tested. In Durango 20 seed production areas have been developed and more than 400 superior trees have been selected. Progeny test seedling orchards will be the core of the Durango program with first generation goals of more than 6000 parents in the tests. Plans are to establish breeding populations of 180-200 parents per seed zone divided into 3 or 4 subpopulations.

Keywords: Pinus arizonica, Pinus durangensis, Pinus engelmannii, seed zones, seed orchards.

INTRODUCTION

Mexico, with 70 or more recognized taxa of Pinus, is extremely rich in representatives of this genus (Dvorak 1987, Eguiluz 1988). Mexican pines were introduced to South Africa early in this century and somewhat later to Australia and New Zealand. Subsequently they have been much used in other parts of Africa and in various countries of Asia, Central and South America and to a lesser extent in Europe (Eguiluz 1987). After the importance of seed origin was recognized, we have within the last 20 years seen a great surge in provenance exploration and testing of species like P. caribaea Morelet, P. oocarpa Schiede and Deppe (Gibson 1987, Picchi 1987), and P. pseudostrobus Lindl. (Pires et al 1987). Since 1980 the CAMCORE Cooperative has also made extensive collections of Mexican pines (Dvorak 1987).

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Several of these species are used in intensive breeding programs in Southern Africa, Australia and Brazil (Gibson 1987) and are the subjects of tree improvement programs in several other countries (Barnes 1988). Thus, the value of Mexican pines is widely recognized outside the country but unfortunately, genetic improvement efforts with pines within Mexico have until recently been very limited. Although the former National Institute of Forestry Research (INIF) during the 1970's established a number of seed production areas and began species and provenance trials, the first major tree improvement programs in Mexico were only initiated after the founding of the Forest Genetics Center (Centro de Genetica Forestal) in 1985.

THE CHIHUAHUA AND DURANGO TREE IMPROVEMENT PROGRAMS

Background and Justification

The forested area of Chihuahua, the largest state in Mexico, is about 5.1 million hectares (12.6 million acres) while that of Durango, the fourth-largest state, amounts to 4.06 million hectares (10.0 million acres). Together, the two states thus contain slightly more than 20 percent of total forest area of the country. The standing volume of timber is estimated to be 259 million m^3 and 280 million m^3, respectively, which represents more than 25 percent of the total volume for Mexico. Conifers, mostly pines, account for about 88.5 percent of the timber volume in the two states. The state of Durango is the leading producer of forest products in the country, followed by Chihuahua and together the wood industries in these states produce about one-half of the total forest products in Mexico. Thus, it is clear that forestry and forest industries are of vital importance for the economy of the states of Chihuahua and Durango.

Forest management practices employed in these states in the past have been largely dysgenic and, as a result, most of the fast-growing high quality trees have already disappeared. While natural regeneration is usually adequate or even abundant in most areas, the problem is, of course, that we are regenerating poor phenotypes. An obvious solution to this problem is to upgrade the stands through the planting of genetically improved stock. Burned-over areas, abandoned farmland and other areas in need of reforestation or afforestation are other logical sites for the introduction of genetically improved materials. Another serious problem in Chihuahua and Durango is the high cost of transportation of the wood from the forests to the mills; up to 60 percent of the total cost of the wood. A partial solution to this problem is the establishment of plantations of genetically improved seedlings in more accesible areas closer to the mills.

The annual planting programs in Chihuahua and Durango have so far been relatively modest. However, Ponderosa Industrial, S.A., one of the largest forest industries in Chihuahua, has recently begun a program aimed at planting 2,000 hectares per year. Estimates are that in order to fulfill the projected needs for commercial plantations in this state, 16,000 hectares should be planted annually.

The situation in Durango is very similar. Three of the 13 Forest Units have small nurseries with a total production of about 1 million plants. The immediate goal is to increase output to 4 million plants, and plans are in the next 6 to 15 years to produce 20 to 25 million plants annually. Production at this level will require the development of a system of probably three central nurseries and several smaller units in the most remote forests.

Considering the economic importance of the forests and the forest industries of Chihuahua and Durango for these states and for Mexico, the need for upgrading of the stands, the need for planting in poorly stocked, burned, cut-over or unforested areas, and the need for plantations closer to the mills, it seemed logical to initiate the first comprehensive Mexican tree improvement programs in those states.

The Forest Genetics Center is responsible for program plans, development of seed zones, evaluation of selections, plans and designs for seed orchards and tests, training of field personnel, and overall coordination of programs and activities. Field personnel carries out selection of superior trees, development of seed production areas, collection of material for seed orchards and various tests and will be responsible for establishment and maintenance of seed orchards and test plantations. Programming has had to take into consideration the total lack of information on the adaptive variation of the priority species in their native environments, the limited investment capacities of the Forest Units, and the lack of trained personnel. The impoverished nature of many of the natural stands and the extreme site variability of Mexican forests have added to the problems. Therefore, the plans, particulary in Durango, are compromises between what needs to be done in the long term and that which the Units can undertake now.

The two programs function independently of each other but work together on problems of joint concern. We plan to cooperate in provenance and progeny testing and, where appropiate, share breeding materials. Due to some differences between the programs they will be discussed separately in the following.

THE CHIHUAHUA PROGRAM

The tree improvement program in Chihuahua was begun in 1987 as a cooperative effort between the federal government, the state government, the nine Forest Administration Units, the wood-using industries, and the Forest Genetics Center.

Goals, Objectives and Priority Species

The goals of the program are to develop and mass-produce genetically improved seed of three priority species of pine for immediate and future use in commercial forest plantations. The specific objectives are: (1) to develop seed zones for each species, (2) to establish a network of seed production areas, (3) to select 1000 superior phenotypes of each species,

(4) to establish three seed orchard complexes, (5) to establish provenance and progeny tests, and (6) to develop plans for long-term selection, breeding and testing. Allthough at least 15 species of pine are native in the state, the program of necessity focuses on the three comercially most important species: P. arizonica Engelm., P. durangensis Martínez, and P. engelmannii Carr.

Accomplishments and Plans

In order to establish some control over the movement of seed and plants within Chihuahua and Durango, we have jointly developed a system of seed zones. Because the forested regions of these states are mountainous the topography is very rough and irregular. Seed zones based on either elevation or mean annual temperature would as a result be extremely complex and impractical to use. Therefore, we used total annual precipitation as the basis for the zones a follows:

Zone	Annual Precipitation (mm)
1	less than 600
2	600 - 800
3	800 - 1000
4	1000 - 1200
5	more than 1200

Chihuahua was divided into three regions based on administrative boundaries with three or four zones within each region or a total of 11 zones in this state. Latitude and administrative boundaries were used to divide Durango into five regions with between two and four zones in each region. Thus there are 5 regions and 17 zones in Durango. We plan to use provenance tests and isozyme studies to verify the validity of these seed zones and following eventual modifications, we plan to develop recommendations for seed transfer within the two states.

In order to meet immediate needs for seed of the three species, we plan to establish 30 seed production areas in Chihuahua. These will be distributed over the three regions and will represent as many seed zones as possible. So far, we have established six areas of P. arizonica, two of P. durangensis, and three of P. engelmannii but hope to double that before the end of the year.

Plans are to select 1000 superior trees of each of the three species, again representing all seed zones where a species is important. Our progress has been modest with 82 P. arizonica, 36 P. durangensis, and 35 P. engelmannii selected to date, but we expect to accelerate the selection progress in the immediate future (Eguiluz and Clausen, 1988).

The 30-50 best selections from each seed zone will be grafted and used to establish seed orchards of each species. We plan to concentrate the individual orchards in three seed orchard complexes, one in each region.

We may also use seedling seed orchards. All of the selections will be progeny tested as soon as we are able to do so.

We still need to determine the best methods of grafting for the three species and to study the phenology of flowering and fruiting for the future breeding work and for management of the seed orchards. We are also planning to study the variation in wood properties of these species. In cooperation with the Durango program we plan to establish a series of provenance tests of the three priority species and of P. cooperi Blanco in the two states. Seed collections for these tests were begun in 1987 with a few collections of P. engelmannii. It now appears 1990 will be the next reasonably good seed year. These tests are our highest research priority.

THE DURANGO PROGRAM

The work in Durango started with one Unit just before the end of 1985, the other twelve Units joined during 1987.

Priority Species

Priority species are the same as in Chihuahua, with the exception that P. cooperi replaces P. arizonica in the three southern regions. Second priority species are P. teocote Schiede and Deppe and P. herrerae Martínez but for these, improvement programs will be limited to the development of seed production areas.

Species priorities have been assigned in each of the 17 seed zones. In two zones work will involve a single species, four zones will include three species and in the 11 remaining zones the program will concentrate on two of the priority species.

Goals, Accomplishments and Plans

Separate breeding populations will be established for each zone, and selection intensity and the type of population will depend on the nature of the stands of the species and investment capacities of the Units. For P. engelmannii, for example, there will be no effort to select superior trees in the conventional sense; the parent trees will be well distributed trees of good quality, but since it rarely is possible to obtain comparison trees, intensive selection will not be used. In some instances selection will be limited to the best trees in the seed production areas.

More than 20 stands have been selected as seed production areas. The work is practically completed in 14 of these and improved seed has been collected in 4. More than 400 superior trees have been selected but over 300 of these are concentrated in a single Unit. The total number of seed production areas will be completed by the end of 1991, and we hope to have the first generation progeny tests established in about six years.

The backbone of the program will be progeny test seedling seed orchards. Clonal seed orchards will be used only in a few instances in some of the Units with the most resources. Some Units will rely on plantations of seed production area seed for the second generation of seed production areas.

The goal is to establish breeding populations of 300-500 parents per species in each seed zone, but this clearly exceeds current resources. Therefore, the majority of the populations will involve 180 to 200 parents; they will be substructured in 3 or 4 subpopulations of 50 or 60 progenies. The first generation goal is the establishment of more than 100 progeny tests with the total number of parents exceding 6000--more than 3500 superior trees and 2500 trees of good quality.

We expect that it will be possible to begin the development of the second generation breeding populations 12 to 15 years after the progeny tests are established. At that time it may be necessary to double the size of the populations with additional selections from natural stands. At the same time the size of subpopulations should be reduced by half. We hope that by then, we will have enough provenance test information to develop realistic zones. Should consolidation of zones be possible, the need for additional selections from natural stands could be eliminated. Any new selections made will be kept in separate subpopulations and we hope we can keep problems of the equalization of the genetic worth of the selections to a minimium.

It should be pointed out that the initial 180 to 200 progenies will be more than adequate for the high intensity selection of second generation short term breeding populations.

Other Center Activities

The Center has conducted several short courses in forest tree improvement and plantation establishment and we also train field personnel in selection of superior trees and in the establishment of seed production areas.

In addition to the Chihuahua and Durango programs, the Center is developing four more in the states of Oaxaca, Mexico, Veracruz and the Federal District, involving eight different pines plus species of Abies and Cupressus.

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Global Tree Improvement Compared to That in the Southern United States

By Bruce Zobel [1]

ABSTRACT

Progress in tree improvement is very rapid throughout the world both in the well established and newly developing programs. Progress is particularly rapid in tropical forestry because of the nature of the species and very short rotations. The only major criticism of the global tree improvement effort is the tendency to "follow-the-leader" whether or not the activity is applicable to the organization involved.

Despite the currently good financial situation in the forest industry, plentiful funds are not available to tree improvement, especially in the Southern U.S., where forestry research is still not generally recognized as being essential. In the tropics, intensive forestry is largely dependent upon exotics where provenance differences and land race development are keys to success; the approach is somewhat different when indigenous species are planted.

Several current areas of emphasis in tree improvement are mentioned. These are vegetative propagation, quantitative genetics and biotechnology, including genetic engineering. Some of the more advanced forestry organizations of the world are criticized for developing, but not thoroughly using, tree improvement principles. It is especially bad where inadequate silvicultural practices do not allow full development of genetic potentials. A case in point is in the Southern U.S. where insufficient investment in good forestry is enabling the newer forest areas in the tropics to become rapidly competitive in wood products. The Southern hemisphere is becoming a major competitor to the Southern U.S. and unless the latter better uses research knowledge, it will suffer. Additionally, new technologies have enabled the production of quality products out of marginal quality wood, thus enabling the tropics and sub-tropics to be even more competitive in many forest product lines. Use of good tree improvement principles will help keep the Southern U.S. competitive.

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INTRODUCTION

I have been greatly impressed by the coverage and the quality of the papers presented at this conference and those I have read from recent conferences. It is good to see this progress which is needed if the Southern United States is to compete with forestry on a world basis. Some tree improvement programs have been in operation for many years including those in Japan, Europe, Australia, South Africa and New Zealand. In fact, considerable work in the South was initially influenced by earlier work done in those countries. Currently, many new programs are developing very rapidly especially in the tropical countries, because of short rotations, early flowering, fast growth and the use of rooted cuttings. Even though much of the work in the tropics was patterned after the older programs, including those in the Southern United States, the tropical programs are developing so rapidly that they will soon pass the older ones unless a major effort is made in the rest of the world to keep ahead.

Two themes will be covered in this paper. The first relates to doing the research and the second is to apply (use) the research in operational forestry. The research reported at this conference is generally well done and has great potential for improving the quality and quantity of forests in the Southern United States, and I am quite complimentary about it. But I am less impressed by the use of the knowledge generated, especially in some silvicultural applications, a deficiency that, unless corrected, will cause the South to lose out to tropical areas.

One criticism, applicable both to the Southern United States and to other parts of the world, is the tendency to "follow-the-leader" and invest in unproven "fads" related to tree improvement. This is often to the extent of overlooking the developmental work needed before more advanced research can be put in practice or before an organization has the ability to do good research with advanced technologies. One finds less of this fascination with current "high-tech fads" in long-term programs such as in most cooperatives, but in any number of instances, they siphon off more than their share of available funds which reduces the amount of support for the more mundane but required studies necessary for tree improvement to be a successful operation.

AN APPRECIATION OF THE VALUE OF FOREST RESEARCH

Currently the forest industries are as well-off financially as they have ever been. Yet, this frequently does not mean an increase in research in forestry, especially in the Southern United States. Forestry in the Southern United States has the dubious honor of having the lowest outlay for research of any major industry with the possible exception of steel, when it should, in fact, spend the most because so little is known and because of the huge gains possible by combining tree improvement, silviculture and utilization technology.

The fact is that the value of forest research is not generally recognized either by foresters or administrators, especially the financial types who think only about term complications engendered by buyouts and mergers have often resulted in withholding of funds for forest research.

How often I have been visiting with a district forester or a general manager when a research report is placed on his desk. Much too frequently, it is glanced at and placed in the wastebasket, often with the comment "I wish they would quit sending me this stuff; it can't be used" (Not the actual quote). The fault is not all with the operational forester. A lot of researchers either don't know or care about the potential utility of their results and present them in a maze of technical language and statistical "gobbledegook" that reduce readability, understanding and acceptance.

On a world basis, there is variation in the acceptance of the need and support for tree improvement activities. Research activities in countries like Australia, New Zealand, South Africa, Korea and in Western Europe are generally well supported. In the Americas the recognition of the importance of tree improvement varies greatly by organization and company, but overall, research is not as well supported as in the countries listed. Frequently, the first two things mentioned when funds for research are sought are "How much does it cost?" and "Give me proof of the economic returns to be obtained". Not much is taken on faith or done with a missionary zeal as was possible in the late 1940's and 1950's when applied tree improvement became seriously supported in the Southern U.S. In the developing countries, and most of the tropical countries there is interest but lack of facilities, trained people or financial support. Much of the research applied in these countries is an adaptation of research done elsewhere, but there are some exceptions (such as in the use of vegetative propagation), where the tropics are leading the world.

When comparing tree improvement of the temperate and tropical parts of the world, the differences in their problems and thus in their approach, must be recognized. Tropical areas have forestry based primarily on exotics so the first job is to determine the best species and provenances to use. Then comes the selection and development of the best land race after which the more conventional breeding can be applied. These initial barriers are great and discouraging to one who wishes to develop his research capabilities quickly. Rapid methods of selecting the best land race do not exist. Some of the most dramatic failures in all forestry have resulted from using short-cuts to assess the best exotics to use. Thus, despite fast growth, short rotations and early flowering, the tree improvement specialist in the tropics does not have the simple nor quick job that many people think.

WHAT IS NEW IN TREE IMPROVEMENT?

It was suggested that I include a section in this paper on new activities in tree improvement. To do this satisfactorily would require a small book. Therefore, I will only mention three of the more important activities with a few words about each. It certainly is necessary to keep up with and to invest in these "new" aspects of tree improvement, but not

to the exclusion of the older and more conventional activities, if tree improvement is to make its full contribution to forestry. The current trend to fund basic or fundamental research and to withhold support from conventional applied research is self defeating. It is necessary that both be done and supportive studies that are necessary must be teamed with the applied studies if the job is to get done.

One activity that is expanding rapidly and using new methodologies is vegetative propagation. Great efforts are being made to use vegetative propagation in operational reforestation, and with considerable success, especially in the tropical and sub-tropical countries. The benefits sought are greater uniformity within the forest and its products and greater utilization of the non-additive genetic variances. Although vegetative propagation is being researched by tree improvers throughout the world, the operational aspects of using rooted cuttings have generally proceeded much more rapidly in the tropical and sub-tropical parts of the world. This is in part due to the characteristics of the species used as well as to some good research and leadership. With many species, particularly the conifers, juvenility, which is necessary for good rooting, good growth and orthotropic form, is lacking. Much good work is being done to develop methods to induce juvenility in trees that have already proven their genetic superiority.

Another activity which has gone on for some time but is now becoming more helpful to the plant breeder, is in the area of quantitative genetics. Much of what has been done by the plant breeder can now be explained and breedomg methods suggested that the tree improver can follow to more efficiently make genetic gains in tree breeding. Especially valuable are crossing designsto reduce relatedness, or to use relatedness, in advanced generation or specialty breeding. Most of the intensive and sophisticated work on quantitative genetics is done where tree improvement has been emphasized for a long time. However, the general concepts are being used effectively in the more recently developed programs.

The third area, "red-hot" now and much emphasized, is what might be termed genetic engineering, including gene transfer and other sophisticated physiological methodologies. There are dangers in overemphasis of these technologies but it is important that they be pursued even though the "when" of operational payoff is not known. After they become successful a whole new horizon will be available for progress in tree improvement. Work of this nature is generally concentrated in the more advanced programs but some of the newer programs are also active unfortunately not always with the necessary quota of highly trained personnel, specialized facilities or sufficient financial support.

BECOMING TOO SOPHISTICATED TOO SOON

There is a tendency throughout the world to follow the new and exciting fads and to skip over the more mundane, and often more routine work needed for successful tree improvement operations. Many researchers, are frequently their bosses, are anxious to be leaders in their field; they try to do this by employing the most daring and sophisticated technologies, many in the high tech area which are not as yet proven operationally. New concepts come along and many people jump on the

"bandwagon" without too much thought as to their proof of success or potential usefulness. For example, what value is a prime method of vegetative propagation, or tissue culture unless one has the genetically improved material with a broad genetic base on which to apply the method or if the species or its wood are not deserved . There is a gap developing - it was evident in this Conference - where sophistication has sometimes exceeded the potential for application. An example is tissue culture - it can't be applied until foresters have learned how to effectively bring the plantlets from the laboratory phase to a plantable stage at a reasonable cost. It reminds me of the early days of rooted cuttings; when I visited many of the earliest programs reporting excellent rooting success I found to my surprise that many rooting studies had been carried only to the callous stage of the cutting and then terminated because the researcher assumed a calloused cutting would automatically root. The same is true with tissue culture - a plantlet in a test tube, (or a top without roots) will not automatically make a plantable tree. Good roots must be formed and the plant conditioned so it is physiologically suitable to grow under forest conditions. Many studies enthusiastically report tissue culture as being successful without the plantlets having been tested under field conditions.

The tendency to become too sophisticated too soon is particularly evident in the new programs, especially those in the developing countries. They often are trying to run before they have learned to walk.

THE APPLICATION OF RESEARCH RESULTS

One criticism of the countries and regions most advanced in tree improvement development is the general slowness of applying results. Often findings ARE NOT USED OPERATIONALLY on a scale needed to stay ahead of the rest of the world. This comment is usually made about forestry in general but it also applies to forest tree improvement. Too often, the genetic research has been done and the improved material may be available, but it cannot express it`s full worth in increased yields, quality and adaptability because of lack of intensive silvicultural application. This lack is because of restricted application of silviculture and not the fault of the tree improver but it certainly reduces the value of the possible genetic gains. Why is it happening that the organizations supporting the research in the United States are not using known successful silvicultural methods to the fullest extent in their operational programs? Regardless of how good the genetic material produced is or the gains could be, they will not be obtained without optimal silviculture. Economic savings are often given by administrative personnel as the reason. "We can't afford that intensive type of silviculture" is a standard comment. A common belief is that labor is too expensive and land is more costly or unavailable in the United States in comparison with other countries. This is, of course, partially true but other forest management expenses can be great in the competitive regions. Although, intensive silviculture is not used generally throughout the world, it is quite commonly found in South America, which is becoming a primary competitor to the Southern United States. The basic problem is that many of the administrators in forestry

in the Southern United States feel they are the "woodbasket of the world" and thus are outcompeting others. They have not made sound assessments of the economic value of the improved tree improvement and silvicultural methods and the need to use them operationally. The fact is that the improved methods are needed if forestry in the Southern United States is to stay competitive.

I'm always challenged with "give me a good example". I could give many examples but one of the best has to do with competition control. There are numerous studies showing added growth (up to doubling) from good competition control within plantations. Yet, after the fairly good site preparation practiced in the Southern United States, the follow-up competition control is frequently poor despite its proven advantages. Nearly all foresters in the Southern Hemisphere know it is essential to use intensive competition control - those that don't simply lose out. Why is it that this one practice well proven to be highly beneficial, is not more widely used in the Southern United States? How many hundreds of times have I heard foresters say "Don't worry about that pine plantation, the trees have their leaders above the brush - they will make it". Certainly they will make it but at what sacrifice? Frequently growth is 1/3 to 1/2 (or even less) of what a free-to-grow plantation will produce. Under these conditions, much of the value from genetic improvement is lost. The only way to obtain maximum genetic gain is to control as many factors that limit growth as possible. There must be a marriage between good genetics and good silviculture if either is to produce anywhere near its potential.

IS THE SOUTHERN HEMISPHERE COMPETITIVE IN FORESTRY WITH THE SOUTHERN UNITED STATES?

The answer is definitely yes! This is in spite of many major problems in forestry in the Southern Hemisphere, both biological and social. One hears and reads about the wonders of forestry in the tropics and many people assume that all that is required is to plant, wait a few years and then harvest. This is generally not true and good forestry in the tropics requires more skills and more intensive management than in the temperate regions of the world. It also requires the development of more specialized genetic stock and land races to withstand the extremes in environments found there. Tolerance to adverse sites and pests must be developed within the exotic species commonly used in tropical forestry. Competition with planted trees is more severe and must be controlled, site preparation is sometimes quite difficult and must be very intensive and provenance and species differences are exacting. Soils are generally poor with often severe nutrient deficiencies so fertilization is essential. But despite these problems, when good forest management is applied and the proper species used, the environmental conditions are such that very fast growth results. It is not unusual in the tropics to harvest Gmelina for pulpwood in four to six years and Eucalyptus are commonly harvested at five to seven years. Pine in the tropics is grown twelve to fifteen years for small sawtimber or for pulpwood and often is thinned at eight years. Thus the initial costs and the intensive management investment in the tropics need not be carried on for long periods. The planted trees capture the site quickly, usually one year or less for Gmelina, one to two years for the eucalypts and two to three years for the pines, because of the rapid

growth. Thus, although initial competition control must be intensive, it does not have to be carried on for a long time period. Selection is sometimes made for dense crowned trees that rapidly shade out the competition.

Another advantage of the tropical and sub-tropical areas is that the genetic response to selection and provenance differences are much more dramatic than at the higher latitudes. Flowering is early and rotations are short so that selection and generation turnover in a genetics program are rapid. The species worked with - the exotic pines, eucalypts, Gmelina and others, have great variability so gains from intensive selection and breeding are large. A great advantage enabling full genetic gains is that most species can be reproduced vegetatively so that non-additive characteristics can be more easily used. Especially important, product uniformity can be great. One cannot overestimate the importance of uniformity. One of the major reasons why eucalypt pulp has become so competitive in the Southern United States, Europe, Asia and even in Canada is the uniformity and reproducibility of the pulp produced. As an example, at Aracruz in Brazil we are using in our vegetative propagation program only those trees with a wood specific gravity range between 0.46 - 0.52 and with high cellulose yields. As a result, the company gets 25% more usuable fiber per unit volume of wood than is obtained from unimproved wood. It is very uniform also.

Until the industry in the Southern United States obtains similar product uniformity, it will be at a disadvantage. One common problem cited in the short rotation tropical exotics is the large percentage of juvenile wood. This is of little importance in the eucalypts and Gmelina, where juvenile wood is not especially poor. It was a serious problem in the pines and at one time it was predicted that we would "drown" in excess juvenile wood. So it seemed until the technologies of TMP, CTMP, oriented strandboard and others have not only made it usuable but desirable for some products. Thus, improved technologies along with good genetic improvements have teamed up to produce products with very acceptable qualities which make tropical forest products competitive with those from the Southern United States. It is urgent to apply everything known in tree improvement if forestry in the Southern U.S. is to stay competitive.

POSTER ABSTRACTS

APPLICATION OF A MERISTEMATIC TISSUE PROLIFERATION SYSTEM TO DIVERSE *PINUS* SPECIES

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A system for *in vitro* propagation will only be useful for large-scale forestry if it produces quality plantlets at an acceptably low cost. The *Pinus radiata* system for meristematic tissue proliferation as developed by Aitken-Christie, particularly if it is automated, may produce the necessary cost reduction to allow efficient clonal forestry with superior conifer material. Several pine species of interest to forestry were investigated for their potential use in the *P. radiata* meristematic tissue proliferation system. The species used are *P. radiata*, *P. oocarpa*, *P. taeda*, *P. caribaea*, *P. eldarica*, *P. tecunumanii*, and *P. strobus*. The pines selected represent species which are taxonomically and morphologically close to and widely divergent from *P. radiata*. Results are presented on how excised mature embryos of these species performed when cultured for three months on the following eight media: full or half-strength Aitken-modified LePoivre medium + 1.0 or 5.0 mg/l benzylaminopurine + 0 or 0.1 mg/l indole-3-butyric acid. Although *P. radiata* performed as expected, no other species exhibited such successful meristematic tissue proliferation. Possible explanations are many and include non-optimal basal medium constituents, growth regulators, and/or environmental conditions. Need for seed stratification and/or pregermination may also vary with species, and this aspect is currently under investigation.

THE DEVELOPMENT OF A CENTER FOR FOREST ENVIRONMENTAL STUDIES

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The stresses that affect forests change constantly and forest managers must decide how to maintain healthy and productive forests when they know these resources may be affected by changes in a variety of environmental parameters; for example, increasing concentrations of tropospheric ozone, increasing sulfate loadings, increasing intensity of ultra-violet light, increasing concentrations of CO_2, increasing temperatures, and increasing frequency and severity of drought. The USDA Forest Service Center for Forest Environmental Studies was built in Macon, Georgia by Forest Pest Management of Region 8 with funding from the Southern Commercial Forest Research Cooperative to provide a state-of-the-art facility that will produce the information foresters need to help them evaluate and deal with these changing environmental stresses, particularly those relating to air pollution. The Center consists of a headhouse, a climate-controlled greenhouse, and laboratory facilities. Existing exposure equipment includes twelve rain tables and twenty Continuously Stirred Tank Reactors. The Center staff will include people with expertise in air pollution, plant physiology, genetics, and soils. The Center will be used to evaluate the effects of, and study the response of tree seedlings to various stresses, to develop procedures for screening families for tolerance to stresses that are found to be significant, and to provide screening services to foresters both inside and outside the Forest Service.

STUDIES ON HIGH DENSITY PLANTING OF FAST GROWING FUEL WOOD TREES

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To study the growth performance of fast growing fuel wood trees grown under different plant densities, the four species viz. Casuarina equisetifolia (Suru), Dalbergia sissoo (Shisoo), Acacia auriculiformis (Australian babhul) and Leucaena leucocephala (Subabhul) were planted in main plots by adopting 1 x 1 m and 2 x 2 m spacings as subplots in split plot design with four replications during kharif (monsoon) 1982. The hilly lateritic soil was having 30-35% slope. It was low in organic matter and macroelements with acidic in reaction (5.8 pH). An average rainfall was 3500 mm receiving from 15th June to 15th September with high humidity and warmer temperatures. Fertilizers @ 100 kg N, 25 kg P_2O_5 and 25 kg K_2O/ha were applied during first year and thereafter this dose was increased by same rate every year and after fifth year; fertilizers @ 500 kg N, 125 kg P_2O_5 and 125 kg K_2O/ha were applied in two splits, first after the onset of monsoon and second at last week of August when the intensity of rain was low.

The maximum growth in relation to height and dbh was observed in Acacia auriculiformis (608 cm in 1 x 1 m spacings and 574 cm in 2 x 2 m spacings) followed by Leucaena leucocephala. The growth of Leucaena was slow up to 21 months after planting. The growth of Dalbergia sisoo was least as compared to other species.

Planting these fast growing fuel wood trees in closer spacing showed more height than by planting at wider spacing, except after 58 months of planting the Casuarina equisetifolia at wider spacing took the lead in height over planting at closer spacing.

All the species showed maximum AGR of height and dbh between 22-33 and 46-57 months after planting, respectively. The wider spacing showed increase in AGR of dbh at later stages of growth, where as closer spacing showed decrease in AGR of dbh from 34-45 months after planting onwards.

Casuarina equisetifolia, Acacia auriculiformis and Leucaena leucocephala are suitable as fast growing fuel wood trees to be planted by adopting closer spacings under the heavy rainfall conditions of Konkan region of India.

EFFECTS OF VESICULAR-ARBUSCULAR MYCORRHIZAE AND SEED SOURCE ON GROWTH OF NURSERY-GROWN JUGLANS NIGRA L. SEEDLINGS

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Twelve Juglans nigra L. seed sources were grown in fumigated nursery soil with and without vesicular-arbuscular mycorrhizal fungi formed by Glomus intraradicies or Glomus etunicatum Becker and Gerd. Nuts were planted on April 30 and seedlings lifted December 20, 1988. Plants inculated with intraradicies held their leaves and retained color longer than those without mycorrhizae or inoculated with G. etunicatum. G. intraradicies appeared to stimulate the greatest amount of fibrous root growth to all seed sources, while G. etunicatum stimulated a greater number of larger primary roots. Data on height, caliper, percent colonization, and number of first order laterals $\geq$ 2 mm are analyzed and discussed.

APPLICATION OF BIOCHEMICAL GENETICS IN NATIONAL FOREST MANAGEMENT

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In 1988, the USDA Forest Service established a national laboratory to assist forest management on the National Forests with application of current biochemical genetics technology. This laboratory provides checks on clonal identity, and controlled cross verification, to help improve accuracy in the management of crossing programs. In seed orchards, isozymes will be useful to estimate pollen contamination and outcrossing rates, as well as to monitor the efficacy of supplemental mass pollination. In natural populations, isozymes will be used to map patterns of genetic variation in natural populations, and to help monitor the effects of management on the gene pool and genetic diversity. In recurrent mass selection programs, inbreeding will be kept to a minimum through the use of isozymes or RFLPs to check selected individuals prior to breeding. Additional projects involve seedlot identification in nursery management, and genetic origin and variation estimates for plantations. Benefits from the lab are expected to be 1) improved decision-making for selecting specific families to include in breeding programs, and 2) additional information on natural populations which can be applied to managed forest tree populations.

THE RIBOSOMAL RNA GENES AND OTHER REPEATED SEQUENCES FROM PINUS RADIATA

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Genes for the 18S and 25S ribosomal RNAs and the 5S RNA have been isolated from a Pinus radiata genomic library. The organization and quantitative variation of these genes have been determined in a series of individual trees. For the 18S and 25S repeat unit, polymorphisms appear between individuals for both the number of genes and their organization. The number of genes in this species can be much lower than that found in angiosperms despite their much larger genomes. However, the repeat unit appears to be much longer, but without a repetitious region in the intergenic region. In situ hybridization has demonstrated that these genes are present on a large number of chromosomes. The organization of the 5S RNA genes appear to be similar to that observed in angiosperms except that again there appear to be fewer copies of this gene family, and these are dispersed over a number of chromosomes. Twenty repetitive sequences have been cloned from the P. radiata genome and used to look for polymorphisms. Unlike the rDNA, these sequences were all from dispersed families and gave no useful quantitative or qualitative markers.

USE OF PRESCRIBED FIRE TO CONTROL THE WHITE PINE CONE BEETLE IN AN EASTERN WHITE PINE SEED ORCHARD

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The white pine cone beetle, Conophthorus coniperda (Schwarz), is one of the most destructive cone and seed insects in North America. Without insecticide application, losses of genetically improved seeds to this pest approach 100% in seed orchards of eastern white pine, Pinus strobus L. Studies in a seed orchard in Cherokee Co., North Carolina, during 1987 and 1988 evaluated the potential of prescribed fire as an alternative to insecticidal control. Four of eight 1-ha experimental plots, along with two large pilot test plots were burned on March 1-3, 1988. Before and after burning, samples of cones on the ground were used to estimate density and mortality of overwintering beetles. Barrier traps were used to monitor spring flight activity of the beetle. Cone attacks were monitored in sample trees in each plot throughout April and early May. Prescribed fire reduced numbers of live overwintering beetles by 99% in the four experimental plots and one pilot test plot. A 90% reduction occurred in the other pilot test plot. Significantly fewer beetles were trapped in burned than in unburned plots. During the first 4 weeks after beetle attacks began in April, there were significantly fewer cone beetle attacks on trees in burned plots than on those in unburned plots. At 6 weeks the difference was still large, but not statistically significant. Late attacks in burned plots appeared to be due to reinfestation from unburned areas of the orchard. Results of our study suggest that fire can be a practical, effective, and economical alternative to insecticides for control of C. coniperda in eastern white pine seed orchards.

USE OF ENZYME Km TO DETERMINE OPTIMAL EXPERIMENTAL TEMPERATURE IN TWO GEOGRAPHICALLY SEPARATED POPULATIONS OF LOBLOLLY PINE

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Mahan and Upchurch (Env. Exp. Bot. 1988. 28:351-357; 28:359-366) have shown that several crop species maintain characteristic leaf temperatures when water and energy inputs are not limiting, provided the dew-point is sufficiently low. Further, they showed a correlation between these characteristic temperatures and the thermal dependence of the Km's of the enzymes they analyzed.

This enzymic approach was used to determine the optimal experimental temperature in loblolly pine. The thermal-metabolic adaptation of two geographically separated populations were assessed from comparison of the thermal dependence of the Km for NADH and oxalacetic acid (OAA) of malate dehydrogenase. Populations from Texas (Texas Superior) and North Carolina (8-76) were tested at temperatures which ranged from 10 to 40°C. Malate dehydrogenase was extracted and purified from needles, and assayed by the disappearance of NADH (at 340 nm). The initial velocities were calculated from the slopes of the traces from a strip chart recorder. The Km's were estimated by a program based on the modified Cornish-Bowden method for the determination of Km. Km for each substrate was plotted as a function of temperature of assay. Presence of an optimal organismal temperature appears as a minimum Km.

THE RELATIONSHIP BETWEEN MORPHOLOGICAL FEATURES AND THE ROOTING OF CUTTINGS OF PINUS CARIBAEA VAR. HONDURENSIS X P. TECUNUMANII

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Over 1400 shoots were collected from young hedged seedlings and dissected into 10 cm lengths, which were set as cuttings. Rooting was examined in relation to clonal and family identity, length of the shoot from which a cutting was dissected, position of the cutting on the shoot, appearance and state of activity of the terminal bud (for terminal cuttings), length of both primary and secondary needles near the tip, at the base, and in the central region of the cutting, and basal diameter.

Regression coefficients for all variables were highly significant for both probability of rooting and the mean number of roots per rooted cutting. Clonal identity was the most effective predictor of both parameters. Of the morphological features, primary needle length was the best predictor of both rooting and the mean number of roots. Shoot selection on the basis of this feature can be used to assure operationally acceptable levels of rooting.

On the basis of trends established for individual morphological features, the type of cutting with the highest probability of rooting would be the terminal segment from a shoot 10 to 19 cm long, with primary needles in excess of 25 mm in length, an active terminal bud, and a basal diameter of 1 to 1.9 mm. Cuttings with the highest probability of rooting tended also to have the highest numbers of roots - for all parameters except basal diameter, for which smaller cuttings (in particular those in the 1 to 1.9 mm class) had fewer roots. Studies are in progress to determine the type of cutting displaying optimal field survival and performance.

TECHNIQUES FOR THE MASS PRODUCTION OF CUTTINGS OF *PINUS CARIBAEA* VAR. *HONDURENSIS*

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Stool plants are established with seed of superior full-sib families. When approximately 30 cm tall (at about 5 months), stool plants are decapitated at 15 to 20 cm. Two months later, the first harvest of cuttings (average over 5 per stool plant) is taken. After another two months, a second harvest of cuttings (average over 15 per stool plant) is available.

After harvest, the shoots are trimmed to 10 cm in length, given a basal dip in 0.5% IBA in 50% ethanol, and set in a 1:1 mixture of peat moss and sand in 170 ml polythene containers. The best setting environment tested for container stock has been a shaded greenhouse with 3 x 1 minute waterings per day.

Using these techniques, average rooting has been in excess of 90%. No *P. caribaea* var. *hondurensis* families displaying a rooting percentage lower than an operationally acceptable level of about 70% have been identified. Cuttings are ready for planting out 7.5 months after setting. The techniques described are potentially useful for the multiplication of limited sexually derived material of superior full-sib families. By adding 12 months to the usual propagation interval (approximately 5.5 months for sowing of seed to availability of planting stock for container seedlings) a multiplication factor of at least 20 (in two harvests of cuttings) can be achieved.

PROPAGATION OPTIONS FOR *PINUS CARIBAEA* VAR. *HONDURENSIS* IN QUEENSLAND, AUSTRALIA

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Technically feasible propagation options which potentially permit the capture of genetic gain greater than that achieved in conventional seed orchards include the propagation of full-sib families via mass artificial pollination in monoclonal orchards, and the propagation of these families by controlled pollination in clone banks followed by multiplication by cuttings. Potentially feasible also is the mass propagation of juvenile cuttings stimulated to develop on superior phenotypes selected at age four.

These propagation strategies are compared with respect to the effects of selection intensity (including the effect of constraints imposed by reproductive phenomena involved in the propagation procedure) and time involved in the propagation procedure.

STIMULATION OF THE DEVELOPMENT OF JUVENILE SHOOTS ON FOUR YEAR OLD TREES OF PINUS CARIBAEA VAR. HONDURENSIS

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Treatments comprising decapitation at "high", "medium" and "low" levels within the crown, combined with heavy pruning of all remaining branches, were applied to unpruned but vigorous edge trees chosen in plantations aged one, four and eight years. Shoots which developed in response to these treatments were collected separately according to level in the crown, assessed morphologically, and set as cuttings.

All decapitation treatments and crown levels were characterized by the development of a significant proportion of shoots with well elongated primary needles, the type of shoot which, if produced on a young hedged seedling, would be expected to root very well.

The rooting of shoots from the lowermost level on the pruned four year old trees (83%) did not differ significantly from that of shoots from the pruned one year old trees. The rooting of shoots from other treatments on the four and eight year old trees was significantly lower.

Continuing studies, which include investigation of stool bed and field performance, are aimed at defining the feasibility of the multiplication, testing and mass propagation of phenotypes selected at age four.

FLOWERING OF GRAFTED RAMETS OF PINUS CARIBAEA VAR. HONDURENSIS IN NORTHERN QUEENSLAND, AUSTRALIA

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The seed cones initiated in the third to sixth years after grafting were counted on each of 266 ramets representing 16 clones in a seed orchard situated at Kennedy, in northern Queensland. There was a rapid increase in the number of flowers produced between the fourth and fifth years after grafting, a typical pattern for P. caribaea var. hondurensis in Queensland. Clonal repeatabilities, and genetic correlations from one season to the next, were generally high.

The planning and design of P. caribaea var. hondurensis orchards in Queensland thus incorporate an expected five year interval between grafting and initiation of the first major crop of seed cones. Orchard strategies aimed at an earlier production of the same total seed requirement would involve the grafting of considerably larger numbers of ramets per clone, and the exclusion of some selected clones.

A TISSUE CULTURE SYSTEM FOR STUDYING DISEASE RESISTANCE TO *PHYTOPHTHORA CINNAMOMI*

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Calli derived from embryos of *Pinus taeda*, *P. echinata*, *P. taeda* x *P. echinata*, and *P. virginiana* were screened for their resistance to *Phytophthora cinnamomi* Rands, the littleleaf disease pathogen. Approximately 50 clones of calli from each of at least two different seed sources of each pine species were selected, except for *P. virginiana* which only had one seed source. The optimum growth condition was established by testing the interactions of calli at different temperatures with growth regulators. A similar condition was determined for hyphae of *P. cinnamomi*. Results showed that a culture medium with 10^{-5} M 2,4-D at 22°C gave best growth of calli and caused the least synergistic reaction between fungus and callus tissue. Three methods were used for evaluating the in vitro resistance reaction: (1) diameter growth of *P. cinnamomi* on the callus surface; (2) amount of intracellular hyphae and cytological change of infected callus cells; and (3) surface reaction of inoculated calli. Loblolly pine was demonstrated to be the most resistant species and shortleaf pine the most susceptible. However, susceptible clones were also found in loblolly pine and loblolly x shortleaf pine hybrids and resistant clones were found in shortleaf pine. Resistance reactions were always inversely correlated with a greater growth rate of *P. cinnamomi* on callus, a larger number of intracellular hyphae, and necrosis and accumulation of phenolic compounds in callus cells.

DEVELOPMENT OF IN VITRO AND IN VIVO SCREENING TECHNIQUES TO DETECT LITTLELEAF DISEASE RESISTANCE

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Two-month-old sand cultured pine seedlings were inoculated with zoospores and chlamydospores of Phytophthora cinnamomi Rands to determine in vivo susceptibility. Seedlings from sand culture were harvested and gently washed with tap water to remove sand particles. Root tips of the intact seedlings were then inoculated with differing dosages of zoospores or chlamydospores of P. cinnamomi in a moist chamber by using a micropipette inoculation technique. Two, five, and ten spores per microliter were used as inoculum dosage treatments.

Susceptibility increased with increasing dosage of both zoospores and chlamydospores for all the species tested. In order from most to least susceptible at the 5 spores/µl dosage were Pinus echinata, P. virginiana, P. taeda, and finally the P. taeda x P. echinata hybrid. Infection rates from chlamydospore inoculation were consistently higher than for zoospore inoculation in all species, indicating that the former have greater inoculum potential.

In vitro inoculation was done by using the same method as described above except that the plantlets were derived from embryonic cotyledons through organogenesis in a tissue culture system. Advantages of the latter include: 1) the elimination of interaction with other microorganisms; and 2) large quantities of genetically identical cloned plantlets were available for inovulation with different inocula and dosages. Preliminary results indicate that P. echinata is more susceptible than P. taeda and the infection rate of root tips was increased with increasing inoculum concentration for all the species tested.

In general, the in vitro inoculation results correlated well with the in vivo results, indicating that this screening method may be suitable for incorporation into a traditional breeding program for littleleaf disease resistance.

INHERITANCE AND LINKAGE ANALYSES OF ENZYME SYSTEMS IN EASTERN COTTONWOOD LEAF TISSUE

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Twenty-eight enzyme systems of Populus deltoides full-sib progenies were resolved by starch gel electrophoresis of expanding leaf tissue extracts. Seventeen enzyme systems were shown to be monomorphic, including first reports (in Populus) for ALD, CTO, FDP, GR, G3PD, MPI, MDR, NADH, PPO and TPI. Of the 11 polymorphic systems studied, the mode of inheritance for ADH, AK-1, AK-4, EST-4, and G6PD were initially determined in this study.

Linkage analyses, conducted on 29 pairs of simultaneously heterozygous loci found in the progenies of five controlled crosses, suggest that AK-1 and EST-4, PER-1 and G6PD, ACO-1 and PGI-2, G6PD and ACO-1, PGI-1 and IDH-2, 6PGD-1 and PGI-1, 6PGD-1 and ACO-1, PER and ADH, PGI-1 and PER-1 are located on different chromosomes and segregate independently. Two linkage groups (ACO-1 and IDH-2; PGI-2 and G6PD) were detected at recombination frequencies of 0.182 and 0.333, respectively. These may be the first linked groups detected in any Populus species.

PRELIMINARY RESULTS OF PROVENANCE TRIALS OF BEEFWOOD (*Casuarina cunninghamiana*) IN CALIFORNIA

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Beefwood (*Casuarina cunninghamiana* Miq.) is a fast-growing evergreen tree native to Australia that is cultivated in California for shelter, fuelwood and erosion control. Like other casuarina species, it can form a symbiosis with the nitrogen-fixing *Frankia* bacteria which enhances the tree's performance on infertile sites. Studies have shown a high degree of variability within the species, both in the native range and overseas planting trials.

Field trials of selected provenances of beefwood were established in 1987 at five sites in northern California. A total of 130 open-pollinated families representing 13 provenances collected from native stands in New South Wales, Australia were planted at three sites of equal experimental design. The objective of these trials is to determine the amount of inherent genetic variation within and between beefwood provenances, and at the conclusion of the trial in 1992 to identify superior individuals and families for inclusion in a clonal seed orchard. In addition, four supplemental trials using bulked provenances were planted to test responses to several environmental and cultural factors: frost, wastewater effluent irrigation, *Frankia* inoculation and selective pre-emergence herbicides.

Early results of the main provenance trials indicate a high degree of variation between and within provenances. Based on height growth at 6 and 12 months after planting, only one provenance (Singleton, NSW) was significantly taller over all three sites. At one year old, the Singleton provenance was 7% taller than the mean height for all provenances over all sites, and about 13% taller than the lowest-ranked provenance. Families within provenances also varied significantly. The tallest families at each of the three sites were from 10 to 20% greater than the mean heights, and from 26 to 44% taller than the slowest-growing families. In a supplemental trial of *Frankia* inoculation, height growth at six months after planting was significantly greater with inoculation in four out of five provenances compared to those not inoculated prior to outplanting.

SOMATIC EMBRYOGENESIS IN BLACK LOCUST

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Black locust (Robinia pseudoacacia L.) is a nitrogen-fixing leguminous forest tree species native to the southeastern United States and extensively cultivated in many parts of the world. Developing seed pods were collected from three black locust trees at weekly intervals from one week following anthesis (May 6) until early fruit maturity (July 8). Developing seeds were excised aseptically from pods and cultured on solid media containing 0, 2 or 4 mg/l 2, 4-dichlorophenoxyacetic acid (2,4-D) and 0 or 0.25 mg/l 6-benzyladenine (6BA). Seeds cultured on media containing hormones remained on these media for either 1 or 3 weeks prior to transfer to hormone-free media, or were cultured on hormone-supplemented media for the entire study. One immature seed explant collected 4 weeks following anthesis produced somatic embryos by direct embryogenesis, following culture for one week on a medium supplemented with 4 mg/l 2,4-D and 0.25 mg/l 6BA and transfer to hormone-free medium. The culture produced approximately 100 somatic embryos, mostly via secondary embryogenesis from the radicles of cotyledon-stage somatic embryos. Most somatic embryos were well-formed, with two cotyledons. Many embryos germinated precociously while on hormone-free medium, producing plantlets that were initially weak. However, most later gained vigor and over 90 percent have produced seedling-like plants following transfer to soil mix and acclimatization to greenhouse conditions.

NEW METHOD ACCURATELY IDENTIFIES PITCH CANKER RESISTANCE IN SLASH PINE AT THE RESISTANCE SCREENING CENTER

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Progeny tests for assessing disease resistance in the field are relatively expensive to establish, must be maintained for several years, and depend on the vicissitudes of infection biology for accurate results. Further, successful disease resistance ranking may interfere with accurate assessments of other attributes important in breeding programs, such as tree growth and form. Greenhouse methods used in the past provided results more quickly and cheaper than field tests, but close control of inoculum exposure was difficult to achieve.

A new greenhouse method was used to evaluate the pitch canker resistance of open-pollinated slash pine families representing a range in relative resistance as determined by field tests. Four-month-old seedlings of 25 families plus a susceptible check family were inoculated by severing the stem just below the bud and spraying the cut surface with a water suspension of Fusarium subglutinans conidia. Seedling responses were evaluated 5, 6 and 7 months after inoculation. Highly significant family differences were observed on all three reading dates, but the best family separation occurred 7 months after inoculation, when family mean infection exceeding a response standard ranged from 2 to 89 percent (mean 48 percent). The susceptible check was always correctly ranked. Field and greenhouse resistance class rankings were nearly identical, and standardized performance scores were highly correlated (r^2 = .71). Moreover, greenhouse performance scores for 13 families screened in earlier developmental tests (conducted 18 months prior with different inocula) and again in this test were highly correlated (r^2 = .84), demonstrating the repeatability of this method. This is despite the fact that many families were from different seed collections and even different seed orchards, and probably had different pollen parents. Operational greenhouse screening of slash pine families for pitch canker resistance can be completed in 6 months from seed sowing at the Resistance Screening Center for the same cost as fusiform rust resistance screening ($100 per seedlot). This method may be useful in establishing specialized breeding orchards for reforestation of pitch canker-prone field sites, in guiding breeding decisions in second generation orchards, and in research applications.

Baruch Forest Science Institute
Best Poster Award Winner

CHARACTERIZATION AND GENETIC CONTROL OF CINNAMYL ALCOHOL DEHYDROGENASE IN LOBLOLLY PINE

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Cinnamyl alcohol dehydrogenase (CAD) is the enzyme which catalyzes the final step of the biochemical pathway which produces coniferyl alcohol, the monomeric precursor of the lignin polymer in softwoods. Two isozymes of CAD are found in loblolly pine. One of these, CAD-2, has very high levels of activity in lignifying xylem using coniferyl alcohol as substrate. CAD-2 also occurs in cambium and both the embryo and megagametophyte of seeds. This isozyme is electrophoretically variable and highly polymorphic in loblolly pine. CAD-2 variants segregate in simple Mendelian ratios and show patterns typical of dimeric enzymes. We have purified CAD-2 approximately 650-fold from germinating loblolly pine seeds. The dimeric structure of the enzyme was confirmed by comparison of the molecular weight estimates for denatured and nondenatured enzyme. Further characterization of the enzyme will include amino acid sequencing, which will provide crucial information needed for cloning the gene for CAD-2. CAD-2 appears to be an enzyme through which the properties of wood could be genetically engineered.

SOMATIC EMBRYOGENESIS IN DOUGLAS-FIR

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Most reports of somatic embryogenesis in gymnosperms have been for Picea, Pinus and Larix; only a few have mentioned Pseudotsuga menziesii. During the last two years we have investigated the requirements for obtaining somatic embryos from immature embryos of Douglas-firs. Development of the zygotic embryos was slower the second season than the first. Erickson and von Arnold's medium, as well as modified Risser and White's medium, and yellow-poplar medium did not stimulate the production of an embryogenic callus. However, modified Brown and Lawrence medium and modified Teasdale medium stimulated the formation of an embryogenic callus. On a basal modified Brown and Lawrence medium somatic embryo development was incomplete, generally stopping prior to the initiation of cotyledons.

Morphological comparisons have been made between the zygotic embryos and similar stage somatic embryos. Overall, development of the two are similar.

Research is continuing on conversion of the somatic embryos to plantlets, the identification of the embryogenic callus type, comparative morphology, and applications to mature embryos.

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SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE MEMBERS

SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE

Membership List (Effective for 1989 annual meeting)

June 1989

GROUP A
(Representatives Appointed for 6-Year Term)

Committee Membership	Term Ends (At End of Annual Meeting)	Representing
Jack A. Pitcher Hardwood Research Council P. O. Box 34518 Memphis, Tennessee 38184-0518 (801) 377-1824	1989	Hardwood Research Council
Marvin Zoerb Union Camp Corporation Woodlands Research P. O. Box 216 Rincon, Georgia 31326 (912) 826-5556	1989	Forest Industry
Siroos T. Jahromi International Paper Company Southlands Experiment Forest Route 1, Box 571 Bainbridge, Georgia 31717 (912) 246-3642	1992	Forest Industry
Mike Waxler Southern Operations Geneticists Weyerhaeuser Corporation Box 1060 Hot Springs, Arkansas 71902 (501) 624-8494	1992	Forest Industry
Garner Barnum Management Forester Arkansas Forestry Commission P. O. Box 4523, Asher Station 3821 W. Roosevelt Road Little Rock, Arkansas 72214 (501) 664-2531	1994	State Forestry Agencies
Russell Pohl Georgia Forestry Commission Box 819 Macon, Georgia 31298-4599 (912) 744-3354	1992	State Forestry Agencies

Committee Membership	Term Ends (At End of Annual Meeting)	Representing
Bill Padgett Alabama Forestry Commission 513 Madison Avenue Montgomery, Alabama 36130-0601 (205) 240-9304	1994	State Forestry Agencies
Charles G. Tauer 08 AG Hall Oklahoma State University Stillwater, Oklahoma 74074 (405) 624-5462	1989	Forestry Schools
(Currently Vacant)	1992	Forestry Schools
David B. Wagner Dept. of Forestry University of Kentucky Lexington, Kentucky 40546 (606) 257-3773	1994	Forestry Schools
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Warren Nance Institute of Forest Genetics Box 2008, GMF Gulfport, MS 39503 (601) 864-3972	1990	Stand Dynamics
Ron Schmidtling Institute of Forest Genetics Box 2008, GMF Gulfport, MS 39503 (601) 864-3972	1992	Racial Variation and Seed Movement
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